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PETITION FEE
Under 37 CFR 1.17(f), (g) & (h)
TRANSMITTAL

(Fees are subject to annual revision)

Send completed form to: Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Application Number	10/677,956
Filing Date	October 1, 2003
First Named Inventor	Suzanne Zebedee
Art Unit	1648
Examiner Name	Zacharlah Lucas
Attorney Docket Number	323-100US D

Enclosed is a petition filed under 37 C.F.R. 1.102(a) **that requires a processing fee (37 CFR 1.17(f), (g), or (h)). Payment of $** 130.00 **is enclosed.**

This form should be included with the above-mentioned petition and faxed or mailed to the Office using the appropriate Mail Stop (e.g., Mail Stop Petition), if applicable. *For transmittal of processing fees under 37 CFR 1.17(i), see form*

Payment of Fees (small entity amounts are NOT available for petition fees)

☒ The Commissioner is hereby authorized to charge the following fees to Deposit Account No. **13-4892** :

☐ petition fee under 37 CFR 1.17(f), (g) or (h) ☒ any deficiency of fees and credit of any overpayments

Enclose a duplicative copy of this form for fee processing.

☒ Check in the amount of $ **130.00** is enclosed.

☐ Payment by credit card (Form PTO-2038 or equivalent enclosed). Do not provide credit card information on this form.

Petition Fees under 37 CFR 1.17(f): Fee $400 Fee Code 1462
For petitions filed under:
§ 1.36(a) - for revocationof a power of attorney by fewer than all applicants.
§ 1.53(e) - to accord a filing date.
§ 1.57(a) - to accord a filing date.
§ 1.182 - for decision on a question not specifically provided for.
§ 1.183 - to suspend the rules.
§ 1.378(e) - for reconsideration of decision on petition refusing to accept delayed payment of maintenance fee in an expired patent.
§ 1.741(b) - to accord a filing date to an application under § 1.740 for extension of a patent term.

Petition Fees under 37 CFR 1.17(g): Fee $200 Fee Code 1463
For petitions filed under:
§ 1.12 - for access to an assignment record.
§ 1.14 - for access to an application.
§ 1.47 - for filing by other than all the inventors or a person not the inventor.
§ 1.59 - for expungement of information.
§ 1.103(a) - to suspend action in an application.
§ 1.136(b) - for review of a request for extension of time when the provisions of section 1.136(a) are not available.
§ 1.295 - for review of refusal to publish a statutory invention registration.
§ 1.296 - to withdraw a request for publication of a statutory invention registration filed on or after the date the notice of intent to publish issued.
§ 1.377 - for review of decision refusing to accept and record payment of a maintenance fee filed prior to expiration of a patent.
§ 1.550(c) - for patent owner requests for extension of time in ex parte reexamination proceedings.
§ 1.956 - for patent owner requests for extension of time in inter partes reexamination proceedings.
§ 5.12 - for expedited handling of a foreign filing license.
§ 5.15 - for changing the scope of a license.
§ 5.25 - for retroactive license.

Petition Fees under 37 CFR 1.17(h): Fee $130 Fee Code 1464
For petitions filed under:
§ 1.19(g) - to request documents in a form other than that provided in this part.
§ 1.84 - for accepting color drawings or photographs.
§ 1.91 - for entry of a model or exhibit.
§ 1.102(d) - to make an application special.
§ 1.138(c) - to expressly abandon an application to avoid publication.
§ 1.313 - to withdraw an application from issue.
§ 1.314 - to defer issuance of a patent.

Signature	FEBRUARY 24, 2006
	Date
JOSEPH E. MUETH, ESQ.	20,532
Typed or printed name	Registration No., if applicable

This collection of information is required by 37 CFR 1.17. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 5 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will very depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U. S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Group Art Unit: 1648
ZEBEDEE et al.	Examining Attorney: Zachariah Lucas
Serial No.: 10/677956	
Filed: October 1, 2003	Date: February 24, 2006 Pasadena, California
For: METHODS AND SYSTEMS FOR PRODUCING RECOMBINANT VIRAL ANTIGENS	

PETITION TO MAKE SPECIAL UNDER 37 C.F.R. §1.102 BASED ON ACTUAL INFRINGEMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. The Applicants hereby petition to make this application special under 37CFR §1.102. This petition is based on actual infringement of at least one claim of the pending application.

2. Statement of Facts:

a. The United States Patent and Trademark Office has conducted at least two separate searches on this subject matter as follows:

(i) Closely related method claims including claim 28 of ultimate parent application United States Patent Application Serial No. 573,643, filed August 27, 1990 (of record in Exhibit 2 to the Declaration of Joseph E. Mueth filed February 13, 2006) was searched by the United States Patent and Trademark Office (see Office Action of April 15, 1992).

(ii) The same general subject matter in the form of claim 35 in United States Patent Application Serial No. 616,369, filed November 21, 1990 (of record in Exhibit 1 of the Declaration of Joseph E. Mueth filed February 13, 2006) was searched again by the United States Patent and Trademark Office, see the Office Action of October 5, 1992.

In addition, the exact subject matter of the presently pending claims 102 to 111 has been searched by the European Patent Office in counterpart European Patent Application No. EP 02 00 6640.3.

All relevant prior art cited by the United States Patent and Trademark Office and the European Patent Office is already of record in this case.

b. The claims of the present application are directed to the method for the early detection of HCV, otherwise referred to as NANAV virus, in a body fluid sample by forming an admixture of the body fluid sample with a NANBV capsid

antigen and allowing immunoreaction to occur to form an immunoreaction product, and detecting the presence of any immunoreaction product formed. The term "core" is the same as "capsid". The term "capsid" is used in the pending claims 102 to 111.

c. The Applicants are aware of at least two commercially HCV immunoassays the sole intended use of which infringe at least one of the instant claims. One infringing immunoassay is sold by Bayer Healthcare (the name of this diagnostic test is "HCV Advia Centaur"). The second infringing assay is sold by Ortho Clinical Diagnostics (the name of this diagnostic test is "Vitros Anti-HCV").

(i) The HCV Advia Centaur package insert (copy attached as Exhibit A to the accompanying Supplemental Declaration of Joseph E. Mueth) states on page 2/20, at the beginning of the summary:

> "The ADVIA Centaur HCV is an indirect two wash sandwich immunoassay used for the detection of IgG antibody to hepatitis C virus (HCV) in human serum or plasma"

and in the last two sentences of the last paragraph of this section it is stated:

> "The c22 peptide is an amino acid sequence derived from the core region of the genome. This peptide contains the major HCV core epitope. An immunological response to the core protein is often an early indicator of infection by HCV."

Page 15/20 in section "Seroconversion Panels" describes the ADVIA assay as being capable of detecting anti-HCV antibodies in seroconversion samples earlier than a reference assay.

(ii) The Vitros Anti-HCV Reagent Pack package insert (Exhibit B attached to the accompanying Supplemental Declaration of Joseph E. Mueth) states on page 3 in section "Summary and explanation of the test":

> "Three recombinant hepatitis C virus encoded antigens are used in the Vitros Anti-HCV assay. The three recombinant antigens are c22-3, c200 and NS5. The recombinant

protein c22-3 is encoded by the putative core region of the HCV genome"

Page 11 of Exhibit B states:

"29 commercially available seroconversion panels were tested. The Vitros Anti-HCV assay showed equivalent or greater seroconversion sensitivity for 29/29 panels..."

d. Enclosed herewith please find a Supplemental Declaration executed by the Applicants' undersigned attorney. The Supplemental Declaration supports the Applicants' position that the pending claims are currently being infringed by the HCV immunoassays identified above.

2. Conclusion:

The Applicants believe that grounds for granting the present petition have been established and hereby respectfully request that the current petition be granted. The

Applicants' undersigned attorney can normally be reached at the telephone number set forth below.

Date: February 24, 2006

Respectfully submitted,

Joseph E. Mueth
Registration No. 20,532

225 South Lake Avenue, 8th Floor
Pasadena, California 91101
Telephone: (626) 584-0396
Facsimile: (626) 584-6862

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Zebedee, Suzanne, et al.**			Docket No. **323-100US D**	
Application No. **10/677,956**	Filing Date **10/01/2003**	Examiner **Zachariah Lucas**	Customer No. **None**	Group Art Unit **1648**

Invention: **METHOD AND SYSTEMS FOR PRODUCING RECOMBINANT VIRAL ANTIGENS**

I hereby certify that the following correspondence:

Petition to Make Special Under 37 C.F.R. 1.102(a) Based On Actual Infringement; Supplemental Declaration of Joseph E. Mueth in Support of Petition to Make Special; Petition Fee Under 37 C.F.R. 1.17(h) Transmittal; Check No. 7246; Certificate of Mailing by "Express Mail"

(Identify type of correspondence)

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on

February 24, 2006
(Date)

SALLY SHORE
(Typed or Printed Name of Person Mailing Correspondence)

Sally V
(Signature of Person Mailing Correspondence)

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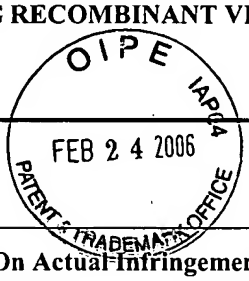
("Express Mail" Mailing Label Number)

Note: Each paper must have its own certificate of mailing.

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): Zebedee, Suzanne, et al.			Docket No. 323-100US D	
Application No. 10/677,956	Filing Date 10/01/2003	Examiner Zachariah Lucas	Customer No. None	Group Art Unit 1648

Invention: METHOD AND SYSTEMS FOR PRODUCING RECOMBINANT VIRAL ANTIGENS



I hereby certify that the following correspondence:

Petition to Make Special Under 37 C.F.R. 1.102(a) Based On Actual Infringement; Supplemental Declaration of Joseph E. Mueth in Support of Petition to Make Special; Petition Fee Under 37 C.F.R. 1.17(h) Transmittal; Check No. 7246; Certificate of Mailing by "Express Mail"

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(Date)

SALLY SHORE
(Typed or Printed Name of Person Mailing Correspondence)

(Signature of Person Mailing Correspondence)

EV 738852815 US
("Express Mail" Mailing Label Number)

Note: Each paper must have its own certificate of mailing.

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): Zebedee, Suzanne, et al.			Docket No. 323-100US D	
Application No. 10/677,956	Filing Date 10/01/2003	Examiner Zachariah Lucas	Customer No. None	Group Art Unit 1648

Invention: METHOD AND SYSTEMS FOR PRODUCING RECOMBINANT VIRAL ANTIGENS

I hereby certify that the following correspondence:

Petition to Make Special Under 37 C.F.R. 1.102(a) Based On Actual Infringement; Supplemental Declaration of Joseph E. Mueth in Support of Petition to Make Special; Petition Fee Under 37 C.F.R. 1.17(h) Transmittal; Check No. 7246; Certificate of Mailing by "Express Mail"

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(Date)

SALLY SHORE
(Typed or Printed Name of Person Mailing Correspondence)

Sally

(Signature of Person Mailing Correspondence)

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("Express Mail" Mailing Label Number)

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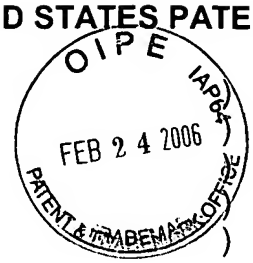
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re Application of:

ZEBEDEE et al.

Serial No.: 10/677956

Filed: October 1, 2003

For: METHODS AND SYSTEMS FOR PRODUCING RECOMBINANT VIRAL ANTIGENS

Group Art Unit: 1648

Examining Attorney: Zachariah Lucas

Date: February 24, 2006
Pasadena, California

SUPPLEMENTAL DECLARATION OF JOSEPH E. MUETH IN SUPPORT OF PETITION TO MAKE SPECIAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Joseph E. Mueth, am counsel of record in the above-identified Patent Application.

1. I am aware of two commercially HCV immunoassays marketed in the United States which, in my opinion, infringe at least one claim of the present application as follows:

(i) Bayer Healthcare immunoassay "HCV ADVIA-Centaur" - Package insert is Exhibit A.

(ii) Ortho Clinical Diagnostics diagnostic test "Vitros Anti-HCV Reagent Pack" - package insert is Exhibit B.

In particular, as illustrated in the Tables to Exhibits A and B attached hereto, each and every element of pending claim 102 can be found in each of the accused immunoassays.

2. It is my opinion that each of the two immunoassays identified above in Paragraph 1 infringe at least one of the claims pending herein.

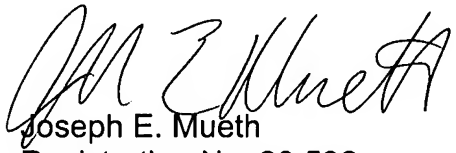
3. All of the relevant prior art in the file histories of United States Patent Application Serial No. 573,643, filed August 27, 1990, United States Patent Application Serial No. 616,369, filed November 21, 1990, United States Patent Application Serial No. 272,271, filed July 8, 1994, United States Patent Application Serial No. 08/931,955, filed September 16, 1997, and in European counterpart Patent Application EP 02 00 6640.3 are of record herein.

I, Joseph E. Mueth, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed

to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

Date: February 24, 2006

Respectfully submitted,



Joseph E. Mueth
Registration No. 20,532

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Pasadena, California 91101
Telephone: (626) 584-0396
Facsimile: (626) 584-6862

EXHIBIT A

TABLE A

Annotated Text of Claim 102	HCV ADVIA-Centaur Immunoassay Package Insert
A method for detecting seroconversion associated with NANBV infection at early times after infection comprising:	Early detection of HCV antibodies by seroconversion, p. 2/20 and p. 15/20
(a) forming an aqueous immunoreaction admixture by admixing a body fluid sample with a NANBV capsid antigen;	"core" antigens which is from capsid and is an NANBV antigen, p. 2/20
(b) maintaining said aqueous immunoreaction admixture for a time period sufficient for allowing antibodies against the NANBV capsid antigen present in the body fluid sample to immunoreact with said NANBV capsid antigen to form an immunoreaction product; and	antigen-antibody form, p. 2/20 last paragraph

(c) detecting the presence of any of said immunoreaction product formed and thereby detecting early seroconversion.	detection, p. 3/20, top paragraph

EXHIBIT B

TABLE B

Annotated Text of Claim 102	Vitros Anti-HCV Reagent Pack Package Insert
A method for detecting seroconversion associated with NANBV infection at early times after infection comprising:	Seroconversion is described at p. 11
(a) forming an aqueous immunoreaction admixture by admixing a body fluid sample with a NANBV capsid antigen;	"core" antigen which is from capsid and is an NANBV antigen, p. 3
(b) maintaining said aqueous immunoreaction admixture for a time period sufficient for allowing antibodies against the NANBV capsid antigen present in the body fluid sample to immunoreact with said NANBV capsid antigen to form an immunoreaction product; and	antigen binds with antibody to form immunoreaction product, p. 4

(c) detecting the presence of any of said immunoreaction product formed and thereby detecting early seroconversion.	Detection by luminescence, p. 4

Bayer HealthCare

QC

CE 0086

HCV

Contents

REF	Contents
03439141	2 vials of Negative Control CONTROL ⊖
	2 vials of Positive Control CONTROL ⊕
	Expected Values Card and barcode labels

Preliminary 00363160 Rev. A, 2004-06

Intended Use

For *in vitro* diagnostic use in monitoring the performance of the HCV assay on the ADVIA Centaur® Systems. The performance of the HCV quality control material has not been established with any other anti-HCV assays.

Control Description

Volume	Ingredients	Storage	Stability
7.0 mL/vial	Processed human plasma negative and positive for anti-HCV with preservatives	2–8°C	Until the expiration date on the vial label or onboard–8 hours

R43 Irritant! May cause sensitization by skin contact. Avoid contact with skin.
S24, S37 Wear suitable gloves. Contains: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one

CAUTION! POTENTIAL BIOHAZARD: The controls contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[1-3] Use eye protection and gloves when handling this product; wash hands after handling.

The negative control has been assayed by FDA-approved methods and found nonreactive for hepatitis B virus, antibody to hepatitis C (HCV), and antibody to HIV-1/2. The positive control has been assayed by FDA-approved methods and found nonreactive for hepatitis B virus and antibody to HIV-1/2. The positive control contains human plasma that is reactive for antibody to HCV. The units were treated with a BPL-UV inactivation procedure, however, all products manufactured using human source material should be handled as potentially infectious.

For *In Vitro* Diagnostic Use.

Preparing the Quality Control Material

Gently swirl and invert the vials to ensure homogeneity.

Using the Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the HCV quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HCV assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 4 to 5 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Reviewing, Editing, and Printing Results

For detailed information about reviewing, editing, and printing quality control results, refer to the system operating instructions or to the online help system.

Expected Results

Refer to the *Expected Values* card for the assigned values specific for the lot number of the HCV quality control material. The expected values are traceable to the standardization of the HCV assay. For additional information, refer to the reagent instructions for use.

The expected values should be used only as a guide in evaluating performance. Since performance is subject to the design and condition of each instrument or reagent system, it is recommended that each laboratory establish its own expected values and acceptable limits. The mean values established should fall within the range specified in *Expected Values*. Individual results may fall outside the range.

Taking Corrective Action

If the quality control results do not fall within the suggested *Expected Values* or within the laboratory's established values, then do the following:

- consider the sample results invalid and repeat testing if controls are out of range
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary contact Bayer HealthCare for more assistance

Limitations

The results obtained using the HCV quality control material depend on several factors. Erroneous results can occur from improper storage, inadequate mixing, or sample handling errors associated with system or assay procedures.

- Do not return any quality control materials back into the vials after testing because evaporation and contamination can occur, which may affect results.
- Dispose of any quality control material remaining in the sample cups after 8 hours.
- Do not refill sample cups when the contents are depleted. If required, dispense fresh quality control materials.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. National Committee for Clinical Laboratory Standards. Procedures for the Handling and Processing of Blood Specimens; Approved guideline-2nd Edition. NCCLS document H18-A2. Wayne (PA):NCCLS;1999.
2. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8.
3. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue; approved guideline. NCCLS Document M29-A2. Wayne (PA):NCCLS;2001.

HCV

Assay for the Detection of Immunoglobulin G (IgG) Antibodies to Hepatitis C Virus

Assay Summary

Sample Type	*Serum, potassium EDTA plasma, lithium or sodium heparinized plasma*
Sample Volume	*10 µL*
Calibrator	*HCV*

Contents

REF	Contents	Number of Tests
03438099	1 ReadyPack® primary reagent pack containing ADVIA Centaur® HCV Solid Phase, Lite Reagent, and Ancillary Reagent	200
	1 Ancillary pack containing ADVIA Centaur HCV Ancillary Reagent [ANC]	
	ADVIA Centaur HCV Master Curve card	
	1 vial HCV Low Calibrator [CAL] L	
	1 vial HCV High Calibrator [CAL] M	
	ADVIA Centaur HCV Calibrator Assigned Value card	

For a definition of symbols used in product labeling, please refer to Appendix D, *Understanding the symbols*, in the *ADVIA Centaur® Assay Manual*.

Intended Use

The ADVIA Centaur HCV assay is an *in vitro* diagnostic immunoassay for the qualitative determination of immunoglobulin G (IgG) antibodies to hepatitis C virus (HCV) in human serum and plasma (EDTA, lithium or sodium heparinized) using the ADVIA Centaur System. The assay may be used in conjunction with other serological and clinical information to aid in the diagnosis of individuals with symptoms of hepatitis and in individuals at risk for hepatitis C infection.

This product is not for use for testing or screening pooled samples containing specimens from more than one individual, or otherwise in blood or plasma screening. Purchase of this product does not convey any right or license under any relevant patents to use the product for testing or screening pooled blood samples containing specimens from more than one individual or otherwise in blood or plasma screening.

WARNING: This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

United States federal law restricts this device to sale by or on the order of a physician.

Materials Required But Not Provided

REF	Description	Contents
	ADVIA Centaur System	
03439141	ADVIA Centaur HCV quality control material	2 x 7 mL Negative Control CONTROL 2 x 7 mL Positive Control CONTROL Expected Value card
01137199 (112351)	ADVIA Centaur Wash I WASH	2 x 1500 mL/pack

Summary and Explanation of the Test

The ADVIA Centaur HCV assay is an indirect two wash sandwich immunoassay used for the detection of IgG antibody to hepatitis C virus (HCV) in human serum or plasma.

HCV is endemic throughout the world and poses a serious health problem. HCV is the major etiologic agent of chronic non-A, non-B hepatitis. The presence of antibodies to HCV indicates that an individual may have been infected with HCV or may be capable of transmitting HCV infection.[1,2]

At least 170 million individuals worldwide are chronically infected with HCV. HCV infection is often asymptomatic; however, the majority (over 55-85%) of the individuals exposed to HCV become chronically infected. In 20% of these chronically infected individuals, the disease progresses to cirrhosis, liver failure, and possibly hepatocellular carcinoma or cholangiocarcinoma.[1-4]

Despite the large number of individuals chronically infected with HCV, the incidence of HCV infections per year in developed countries has declined significantly over the last few decades. This has been attributed to improved standards of living and increased public health measures in the medical community such as the screening of blood and blood products, the use of disposable syringes and needles, and the implementation of universal precautions throughout the healthcare system.[2]

Common modes of HCV transmission include blood transfusion, intravenous drug use, nosocomial exposure, during assisted reproductive techniques, and from mother-to-infant during pregnancy, delivery, or the postpartum period.[2,5]

The HCV genome consists of several functional regions: the core, the envelope (including the E1 and E2 regions), and the non-structural region (including NS2, NS3, NS4, and NS5). Immunoassays for the detection of antibodies to HCV utilize a combination of synthetic or recombinant proteins as antigens.[1]

The ADVIA Centaur HCV assay uses two HCV recombinant (c200 and NS5) antigens and one synthetic HCV core (c22) peptide. The c200 protein is derived from both the NS3 and NS4 sequences. At least two major epitopes are located within the NS3 and NS4 regions. These two specific epitopes have been extensively studied and shown to be critical for the detection of antibodies in individuals infected with HCV. The NS5 antigen is derived from the putative RNA polymerase portion of the HCV genome. A significant number of individuals infected with HCV develop an immunologic response to NS5. The c22 peptide is an amino acid sequence derived from the core region of the genome. This peptide contains the major HCV core epitope. An immunologic response to the core protein is often an early indicator of infection by HCV.[1]

Assay Principle

The ADVIA Centaur HCV assay is an indirect two wash sandwich immunoassay. The sample is incubated with Solid Phase containing recombinant and synthetic peptide HCV antigens. Antigen-antibody complexes will form if anti-HCV antibody is present in the sample. Lite

Reagent containing monoclonal anti-human IgG labeled with acridinium ester is used to detect anti-HCV IgG in the sample.

The system automatically performs the following steps:

- dispenses 10 µL of sample into a cuvette
- dispenses 100 µl Ancillary Reagent and incubates for 5 minutes at 37°C
- dispenses 100 µL of Solid Phase reagent and 50 ul of Ancillary Reagent and incubates for 18 minutes at 37°C
- separates the Solid Phase from the mixture and aspirates the unbound reagent
- washes the cuvette with Wash 1
- dispenses 50 µL of Lite Reagent, incubates the mixture for 18 minutes at 37°C
- washes the cuvette with Wash 1
- dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system

The relative light units (RLUs) detected by the ADVIA Centaur system are used to calculate the Index Value from the Master Curve. Assay results above the cutoff of the assay are not indicative of antibody level. Refer to *Interpretation of Results* for a description of the Cutoff Value calculation.

Specimen Collection and Handling

Serum, potassium EDTA plasma, lithium or sodium heparinized plasma are the recommended sample types for this assay.

Do not use specimens with obvious microbial contamination. The performance of the ADVIA Centaur HCV assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma such as saliva, urine, amniotic fluid, or pleural fluid.

The following general recommendations for handling and storing blood samples are furnished by the National Committee for Clinical Laboratory Standards[6], and augmented with additional sample handling studies using the ADVIA Centaur HCV assay:

- Handle all samples as if capable of transmitting disease.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells. The centrifugation step may occur up to 24 hours post draw.
- Test samples as soon as possible after collecting. Store samples at 2 to 8°C if not tested immediately.
- Store samples stoppered and upright at all times at 2 to 8°C up to 7 days.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage. Do not store in a frost-free freezer. When 10 samples were subject to 4 freeze/thaw cycles, no clinically significant differences were observed. Thoroughly mix thawed samples and centrifuge at 10,000g for 2 min before using.
- Package and label samples for shipment in compliance with applicable federal and international regulations covering the transport of clinical samples and etiological agents. Samples refrigerated up to 7 days demonstrated no qualitative differences. Store samples stoppered and upright at 2 to 8°C upon arrival. If shipment is expected to exceed 7 days, ship specimens frozen.

Before placing samples on the system, ensure the following:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation. (example: 1500xg for 10 minutes; follow tube manufacturer's recommendations[6])
- Samples are free of bubbles or foam.

Reagents

Store the reagents upright at 2–8°C.

Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents*.

Keep away from sunlight. Protect reagent packs from all light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2–8°C away from light sources.

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIA Centaur HCV ReadyPack primary reagent pack	Lite Reagent	10.0 mL/ reagent pack	anti-human IgG monoclonal antibody (~0.05 µg/mL) labeled with acridinium ester in buffer with bovine serum albumin, sodium azide (< 0.1%), and surfactant	2–8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Solid Phase	20.0 mL/ reagent pack	streptavidin coated paramagnetic microparticles preformed with biotinylated recombinant c200 HCV antigen and biotinylated synthetic c22p HCV antigen (~0.3 µg/mL) in buffer with surfactant, stabilizers, and preservatives	2–8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Ancillary Reagent	10.0 mL/ reagent pack	biotinylated recombinant NS5 HCV antigen (~0.5 µg/ mL) in buffer with sodium azide (< 0.1%), and surfactant	2–8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
ADVIA Centaur HCV Ancillary Reagent Readypack	Ancillary Reagent	20.0 mL/ Ancillary pack	bovine serum albumin, goat serum, , sodium azide (< 0.1%), and surfactant	2–8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
HCV calibrator vials	Calibrators	2.0 mL/ vial	processed human plasma negative and positive for anti-HCV with preservatives	2–8°C	until the expiration date on the vial or onboard–8 hours
HCV quality control material vials*	Controls	7.0 mL/ vial	processed human plasma negative and positive for anti-HCV with preservatives	2–8°C	until the expiration date on the vial or onboard–8 hours
ADVIA Centaur Wash 1*	Wash 1	1500 mL/ pack	phosphate buffered saline with sodium azide (< 0.1%) and surfactant	2–25°C	until the expiration date on the vial or onboard–14 days

* See Materials Required But Not Provided

Precautions and Warnings

For *In Vitro* Diagnostic Use.

CAUTION: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

R43 S24 S37 **Irritant!** May cause sensitization by skin contact. Avoid contact with skin. Wear suitable gloves. **Contains:** 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one; included in Calibrators and Controls.

CAUTION! POTENTIAL BIOHAZARD: Some components of this product contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[7-9]

The negative control has been assayed by FDA-approved methods and found to be nonreactive for hepatitis B virus, antibody to HCV, and antibody to HIV-1/2. The positive control, low calibrator and high calibrator have been assayed by FDA-approved methods and found to be nonreactive for hepatitis B virus and antibody to HIV-1/2. The positive control, low calibrator, and high calibrator contain human plasma that is reactive for antibody to HCV. The units were treated with a BPL-UV inactivation procedure[10], however, all products manufactured using human source material should be handled as potentially infectious.

Loading Reagents

Ensure that the system has sufficient primary reagent and ancillary packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

CAUTION: Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents* in the ADVIA Centaur Assay Manual.

Load the ReadyPack primary reagent packs in the primary reagent compartment using the arrows on the packs as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. Load the ReadyPack ancillary reagent packs in the ancillary reagent entry. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

CAUTION: The Low and High Calibrators provided in this kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

CAUTION: The Ancillary Reagent provided in this kit is matched to the ReadyPack primary reagent pack. Do not mix Ancillary Reagent lots with different lots of reagent packs.

Onboard Stability and Calibration Interval

Onboard Stability	Calibration Interval
41 days	28 days

The ADVIA Centaur HCV assay requires a two-point calibration:

- when changing lot numbers of primary reagent packs
- when replacing system components
- when quality control results are repeatedly out of range

CAUTION:

- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

Master Curve Calibration

The ADVIA Centaur HCV assay requires a Master Curve calibration when using a new lot number of Lite Reagent, Solid Phase, and Ancillary Reagent. For each new lot number of Lite Reagent, Solid Phase, and Ancillary Reagent, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

Calibration

For calibration of the ADVIA Centaur HCV assay, use ADVIA Centaur HCV Calibrators provided with each kit. The calibrators provided in this kit are matched to the ReadyPack primary reagent pack.

Using Barcode Labels

NOTE: Calibrator barcode labels are lot number specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur HCV Calibrator barcode labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur HCV assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard. For detailed information about entering calibrator values, refer to the system operating instructions or to the online help system.

NOTE: This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.

1. Schedule the calibrators to the worklist.
2. Label two sample cups with calibrator barcode labels: one for the low and another for the high.

NOTE: Each drop from the calibrator bottle is approximately 50 µL.

3. Gently mix the Low and High Calibrators and dispense at least 4 to 5 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any calibrator remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Quality Control

For quality control of the ADVIA Centaur HCV assay, use ADVIA Centaur HCV quality control materials. Refer to the Expected Value card for the suggested expected values specific for the lot number of the positive and negative controls. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTES: The quality control material furnished is intended to monitor substantial reagent failure. If additional controls are desired, it is recommended to run a negative control and positive control close to the clinically relevant point (1.0 Index). The quality control furnished is in a defibrinated plasma, e.g., serum matrix. The user should provide alternate control material for plasma when necessary.

Using Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur HCV quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HCV assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 4 to 5 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Taking Corrective Action

If the quality control results do not fall within the suggested Expected Values or within the laboratory's established values, then do the following:

- consider the sample results invalid and repeat testing if controls are out of range.
- investigate and determine the cause for the unacceptable control results

- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare.
- verify that the materials are not expired.
- verify that required maintenance was performed.
- if necessary contact Bayer HealthCare for more assistance.
- When the condition is corrected, retest the controls and confirm that results are within acceptable limits.
- It is advisable to repeat some or all patient specimens before reporting results for this run.

Sample Volume

This assay requires 10 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the *ADVIA Centaur Reference Manual.*

Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

CAUTION: Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Prepare the sample container for each sample, and place barcode labels on the sample containers, as required.
2. Load each sample container into a rack, ensuring that the barcode labels are clearly visible.
3. Place the racks in the entry queue.
4. Ensure that the assay reagents are loaded.
5. Start the entry queue, if required.

Procedural Notes

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all country and local requirements.

Interpretation of Results

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

The system reports anti-HCV antibody results in Index Values and as reactive, nonreactive, or equivocal. Index values above the cutoff of the assay are not indicative of the antibody level present in the sample.

- Samples with a calculated value of less than 0.80 Index Value are considered nonreactive (negative) for IgG antibodies to HCV.

- Samples with a calculated value greater than or equal to 0.80 Index Value and less than 1.00 Index Value are considered equivocal. It is recommended that the sample be repeated in duplicate. If 2 of the 3 sample results are less than 0.80 Index Value, the sample is considered nonreactive. If 2 of the 3 sample results are greater than or equal to 1.00 Index Value, the sample is considered reactive and supplemental testing of the sample is recommended. If 2 of the 3 sample results are greater than or equal to 0.80 Index Value and less than 1.00 Index Value, supplemental testing of the sample is recommended.
- Samples with a calculated value greater than or equal to 1.00 Index Value are considered reactive for IgG antibodies to HCV. Supplemental testing of the sample is recommended.
- The Supplemental testing algorithm is described in the Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus, MMWR 2003: 52(RR03) from the Centers for Disease Control.
- Sample results are invalid and must be repeated if the controls are out of range.

Limitations

- The ADVIA Centaur HCV assay is limited to the detection of IgG antibodies to hepatitis C virus in human serum or plasma (potassium EDTA, lithium or sodium heparinized plasma).
- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to hepatitis C virus.
- The calculated values for hepatitis C in a given specimen as determined by assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with different assay methods cannot be used interchangeably. The reported antibody level cannot be correlated to an endpoint titer.
- The performance of the assay has not been established for populations of immunocompromised, immunosuppressed, infants, children, or adolescent patients.
- Assay performance characteristics have not been established when the ADVIA Centaur HCV assay is used in conjunction with other manufacturers' assays for specific HCV serologic markers.
- The performance of the ADVIA Centaur HCV assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic fluid, or pleural fluid.
- Do not use specimens with obvious microbial contamination.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[11] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- A reactive anti-HCV result does not exclude co-infection by another hepatitis virus.

Expected Results

Approximately 61.21% (1335/2181) of the study subjects in the ADVIA Centaur HCV clinical study reported no recent or current signs or symptoms of hepatitis. The asymptomatic population included patients at high risk of HCV infection due to lifestyle, behavior, occupation, disease state (eg, infection with HIV, transplant recipient, dialysis patient, and hemophilia), or due to known exposure events. In the asymptomatic population, 469 of 1335 patients (35.13%) were tested at the Florida site, 445 of 1335 patients (33.33%) were tested at the Texas site, and 421of 1335 patients (31.54%) were tested at the New York site. The asymptomatic study population was 40.30% Caucasian, 26.30% Hispanic, 26.14% African American, 2.92% Asian, and 4.34% from unknown or other ethnicity. The majority of patients in the asymptomatic population were male (52.88% male and 47.12% female). The mean age in the asymptomatic population was 41.8 years (range of 12 to 82 years).
A total of 435 of 1335 patients (32.58%) in the asymptomatic population were reactive in the ADVIA Centaur® HCV assay. (Samples were considered reactive if they were reactive in the ADVIA Centaur® HCV assay upon repeat testing). For the asymptomatic population, the following percentages of patients at each testing site had reactive ADVIA Centaur® HCV results: 32.41% at Florida, 23.15% at Texas, and 42.76% at New York.

The table below summarizes the distribution of ADVIA Centaur reactive and nonreactive results among the asymptomatic population, by age and gender.

Bayer ADVIA Centaur® HCV Assay
Distribution of Asymptomatic Population by Age Group and Gender
All Testing Sites

Age (Years)	Gender	Reactive[a]		Equivocal[b]		Nonreactive[c]		Total
		N	%	N	%	N	%	N
0-9	Male	0	–	0	–	0	–	0
	Female	0	–	0	–	0	–	0
	Overall	0	–	0	–	0	–	0
10-19	Male	7	58.33	0	–	5	41.67	12
	Female	0	–	0	–	14	100.00	14
	Overall	7	26.92	0	–	19	73.08	26
20-29	Male	52	49.52	0	–	53	50.48	105
	Female	11	11.46	1	1.04	84	87.50	96
	Overall	63	31.34	1	0.50	137	68.16	201
30-39	Male	42	27.63	0	–	110	72.37	152
	Female	30	20.69	0	–	115	79.31	145
	Overall	72	24.24	0	–	225	75.76	297
40-49	Male	94	46.31	1	0.49	108	53.20	203
	Female	58	32.95	2	1.13	116	65.91	176
	Overall	152	40.11	3	0.79	224	59.10	379
50-59	Male	67	47.52	0	–	74	52.48	141
	Female	34	30.91	0	–	76	69.09	110
	Overall	101	40.24	0	–	150	59.76	251
60-69	Male	14	22.58	0	–	48	77.42	62
	Female	15	23.08	0	–	50	76.92	65
	Overall	29	22.83	0	–	98	77.17	127
≥70	Male	6	19.35	0	–	25	80.65	31
	Female	5	21.74	0	–	18	78.26	23
	Overall	11	20.37	0	–	43	79.63	54
Unknown	Male	0	–	0	–	0	–	0
	Female	0	–	0	–	0	–	0
	Overall	0	–	0	–	0	–	0
Total	Male	282	39.94	1	0.14	423	59.92	706
	Female	153	24.32	3	0.47	473	75.20	629
	Overall	435	32.58	4	0.29	896	67.12	1335

a Samples with an Index Value ≥ 1.00
b Samples with an Index Value ≥ 0.80 and <1.00
c Samples with an Index Value < 0.80

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[12]

Performance Characteristics

Clinical Performance

To evaluate the ability of the ADVIA Centaur HCV assay to detect anti-HCV antibody in a group of individuals that would normally be tested in a clinical situation a multi-center prospective study was conducted. A total of 2181 patients were tested. Of these 2181 patients, 1335 patients (61.21%) were from patients considered at risk for hepatitis (the high risk population) due to lifestyle, behavior, occupation, disease state (eg, infection with human immunodeficiency virus [HIV], transplant recipient, dialysis patient, and hemophilia), or due to known exposure events. A total of 846 patients (38.79%) were from individuals exhibiting signs and/or symptoms of hepatitis infection (the signs and symptoms population). The prospective study population was 44.66% Caucasian, 25.81% Hispanic, 22.24% African American, 3.12% Asian, and 4.17% from unknown or other ethnicity. The majority of patients were male (55.62% male and 44.38% female). The mean age was 45.4 years (range of 12 to 82 years). Patients in the prospective study population were from the following geographic regions: Florida (37.09%), Texas (32.10%), New York (19.58%), California (7.79%), and 3.44% were from an unknown or other geographic region.
The HCV status for each patient was determined from results of a reference assay for the detection of anti-HCV and the Chiron® RIBA® HCV 3.0 SIA.
Testing for anti-HCV using the reference method and the ADVIA Centaur® HCV assay were performed at each of 3 testing sites (Florida, Texas, and New York). Patients were enrolled at the 3 testing sites and 1 additional clinical site..

Results by Specimen Classification

Following testing with the reference anti-HCV assay and supplemental testing with the Chiron® RIBA® HCV 3.0 SIA where indicated, 2141 subjects were assigned an HCV status of HCV infected or not HCV infected based on the final results obtained with both assays as required. The HCV status of the remaining 40 subjects could not be determined due to indeterminate results with the Chiron® RIBA® HCV 3.0 SIA. Assignment of HCV status is presented in the following table.

Reference Anti-HCV Assay Result	Chiron® RIBA® HCV 3.0 SIA Result	HCV Status
Nonreactive	Not Applicable	**Not Infected**[a]
Repeatedly Reactive	Positive	**Infected** State or Associated Disease Not Determined
Repeatedly Reactive	Negative	**Not Infected**[a]
Repeatedly Reactive	Indeterminate	**Not Determined** HCV Status Cannot be Determined

a. A negative test result does not exclude the possibility of exposure to hepatitis C virus.

Comparison of Results

The following table compares Centaur HCV results with HCV status according to a ranking of the risk of HCV infection in study subjects (N=2181). The ranking was based on a clinical evaluation of the chances of acquiring the disease through the following modes of transmission, with the most common given higher rankings. Each patient was assigned only one risk (the highest). Assignment of HCV status was according to the algorithm presented in the previous table.

Bayer ADVIA Centaur® HCV
HCV Status and ADVIA Centaur® HCV Assay Results for the Prospective Population by Presumptive Diagnosis and Risk Groups for Hepatitis
ADVIA Centaur® HCV Assay vs. HCV Status
All Testing Sites

Presumptive Diagnosis and Risk Groups	HCV Status[a]									
	Infected			Not Determined			Not Infected			
	ADVIA Centaur® HCV Assay[b]			ADVIA Centaur® HCV Assay			ADVIA Centaur® HCV Assay			
	Reactive	Equivocal	Non-reactive	Reactive	Equivocal	Non-reactive	Reactive	Equivocal	Non-reactive	
	N	N	N	N	N	N	N	N	N	Total[c]
Signs and Symptoms	632	0	0	23	0	1	5	0	185	846
Hemophiliac	77	0	0	1	0	0	0	0	3	81
Intravenous drug user, current or past	192	0	0	4	0	0	3	0	55	254
Dialysis	30	0	0	5	0	0	2	0	163	200
Transfusion/ Trans-plant	66	0	0	1	0	0	0	2	172	241
High Risk Sex[d]	21	0	0	4	0	0	1	1	197	224
Healthcare Worker	8	0	0	0	0	0	0	0	201	209
HIV Infected	1	0	0	0	0	0	0	0	10	11
Other[e]	17	0	0	1	0	0	1	1	95	115
None Specified	0	0	0	0	0	0	0	0	0	0
Overall	1044	0	0	39	0	1	12[f]	4[g]	1081	2181

a Final HCV status was based on the reference test results and Chiron® RIBA® HCV 3.0 SIA supplemental testing of samples that were repeatedly reactive by reference anti-HCV assay testing.

b Final ADVIA Centaur® HCV status was based on the initial test result and retest of initially reactive samples.

c Total number of test results by risk population.

d The high risk sex group includes patients with a diagnosis of a sexually transmitted disease, a sexual partner with a history of hepatitis, same sex sexual preference, multiple sex partners, HIV infected partner, or prostitutes.

e The Other risk group includes patients with the following risk factors: sharing straw cocaine, tattoo, history of incarceration, body piercing, family history of hepatitis, immunocompromised patient, tattoo artist, mortician, or other known hepatitis exposure event.

f These 12 patients had nonreactive results in the reference anti-HCV assay and/or had negative results in the Chiron® RIBA® HCV 3.0 SIA assay and were considered to be HCV not infected. Eleven of these 12 patients had reactive results in the ADVIA Centaur® HCV assay, 4 patients had reactive results in both the ADVIA Centaur® HCV and the reference anti-HCV assays, and 1 patient had nonreactive results in the ADVIA Centaur® HCV assay. (Although this patient was initially nonreactive in the ADVIA Centaur® HCV assay, retest Index Values were reactive [> 1.0] and the patient was considered to have a reactive result in the Centaur® method).

g These 4 patients had nonreactive results in the reference anti-HCV assay and/or had negative results in the Chiron® RIBA® HCV 3.0 SIA assay and were considered to be HCV not infected. All of these 4 patients had equivocal results in the ADVIA Centaur® HCV assay.

Note The data described in footnotes *f* and *g* did not result in any modifications to the calculations of percent agreement described below.

The HCV status of 40 patients in the prospective population was considered to be not determined. Samples from these patients were repeatedly reactive in the reference anti-HCV assay and were indeterminate in the Chiron® RIBA® HCV 3.0 SIA assay. Additional testing for these patient samples was performed by using the COBAS AMPLICOR® HCV test, version 2.0 (COBAS AMPLICOR HCV-NAT). Results of the ADVIA Centaur® anti-HCV testing, COBAS AMPLICOR HCV-NAT supplemental testing, and HCV status as determined by HCV-NAT testing are shown in the following table.

Bayer ADVIA Centaur® HCV
NAT Supplemental Testing of HCV Status Not Determined Specimens
ADVIA Centaur® HCV Assay vs. HCV Status
All Testing Sites

Centaur® HCV Assay Results	*COBAS – Amplicor® HCV-NAT*[a]	*HCV Status Following HCV-NAT Test*	*Number of Samples*	*Presumptive Diagnosis and Risk Groups*
Reactive[b]	*Detected (P)*	Infected	19	*Signs and Symptoms*
			1	Hemophiliac
			2	IVDU, current or past
			1	Dialysis
			1	Transfusion/Transplant
			3	High Risk Sex
Reactive[c]	*Not Detected (N)*	Not determined	4	*Signs and Symptoms*
			2	IVDU, current or past
			4	Dialysis
			1	High Risk Sex
			1	Other
Negative[d]	Not Detected (N)	Not Determined	1	Signs and Symptoms
Total			40	

a Subjects found to have indeterminate HCV status by Chiron® RIBA® 3.0 SIA.

b On the basis of detection of HCV by COBAS AMPLICOR® HCV-NAT testing, an accurate laboratory diagnosis of HCV infected was made for these 27 patients who were classified as HCV not determined due to positive results of reference anti-HCV testing and indeterminate results of Chiron® RIBA® HCV 3.0 SIA testing. The reactive Centaur® HCV result was presumed to be correct (true positive).

c Because HCV was not detected by COBAS AMPLICOR® HCV-NAT testing, an accurate laboratory diagnosis of HCV status was not possible for these 12 patients who were classified as HCV not determined due to positive results of reference anti-HCV testing indeterminate results of Chiron® RIBA® HCV 3.0 SIA testing. The reactive Centaur® HCV result was presumed to be incorrect.

d Because HCV was not detected by COBAS AMPLICOR® HCV-NAT testing, an accurate laboratory diagnosis of HCV status was not possible for this patient who was classified as HCV not determined due to positive results of reference anti-HCV testing and indeterminate results of Chiron® RIBA® HCV 3.0 SIA testing. The nonreactive Centaur® HCV result was presumed to be incorrect.

Percent Agreement

Percent positive and percent negative agreement between the Centaur HCV assay and HCV status were calculated for subjects with various risks for viral hepatitis or HCV infection, and for the overall study population (N=2181). The table below summarizes these calculations and provides the upper and lower 95% exact confidence intervals. For the purposes of calculating percent agreement, Centaur HCV assay reactive samples whose HCV status remained 'Not Determined' following supplemental NAT testing were considered 'Not HCV Infected' , and Centaur HCV assay nonreactive samples whose HCV status remained 'Not Determined' following supplemental NAT testing were considered 'HCV Infected'.

Bayer ADVIA Centaur® HCV
Percent Agreement and Confidence Intervals for Infected and Not Infected HCV Status and ADVIA Centaur® anti-HCV Assay: Positive and Negative Results by Presumptive Diagnosis and Risk Groups for Hepatitis ADVIA Centaur® HCV Assay vs. HCV Status
All Testing Sites

Presumptive Diagnosis and Risk Groups	*Positive Percent Agreement % (x/n)[a]*	*95% Exact Confidence Interval*	*Negative Percent Agreement % (x/n)[a]*	*95% Exact Confidence Interval*
Signs and Symptoms	99.85 (651/652)	99.15 to 100.00	95.36 (185/194)	91.38 to 97.86
Hemophiliac	100.00 (78/78)	95.38 to 100.00	100.00 (3/3)	29.24 to 100.00
IVDU, current or past	100.00 (194/194)	98.12 to 100.00	91.67 (55/60)	81.61 to 97.24
Dialysis	100.00 (31/31)	88.78 to 100.00	96.45 (163/169)	92.43 to 98.69
Transfusion/ Transplant	100.00 (67/67)	94.64 to 100.00	98.85 (172/174)	95.91 to 99.86
High Risk Sex[b]	100.00 (24/24)	85.75 to 100.00	98.50 (197/200)	95.68 to 99.69
Healthcare Worker	100.00 (8/8)	63.06 to 100.00	100.00 (201/201)	98.18 to 100.00
HIV infected	100.00 (1/1)	2.50 to 100.00	100.00 (10/10)	69.15 to 100.00
Other[c]	100.00 (17/17)	80.49 to 100.00	96.94 (95/98)	91.31 to 99.36
None Specified	--	--	--	--
Overall	99.91 (1071/1072)	99.48 to 100.00	97.48 (1081/1109)	96.37 to 98.32

a x = the number of ADVIA Centaur® HCV results that were reactive (or were nonreactive) in agreement with the final HCV status as determined by supplemental testing where necessary; n = the total number of final HCV infected status (or final HCV not infected status) results as determined by supplemental testing, where necessary.
Positive/negative % agreement = {[Number of ADVIA Centaur® HCV reactive (confirmed) or non-reactive in agreement with the HCV infected status or HCV not infected HCV status / [Total number of HCV infected status or HCV noninfected HCV status]} X 100.

b The high risk sex group included patients with a diagnosis of a sexually transmitted disease, a sexual partner with a history of hepatitis, same sex sexual preference, multiple sex partners, HIV infected partner, or prostitutes.

c The other risk group includes patients with the following risk factors: sharing straw cocaine, tattoo, history of incarceration, body piercing, family history of hepatitis, immunocompromised patient, tattoo artist, mortician or other known hepatitis exposure event.

The overall positive percent agreement between the ADVIA Centaur® HCV assay results and HCV infected status for the prospective population was 99.91% (1071 of 1072 patients). The overall negative percent agreement between the ADVIA Centaur® HCV assay results and HCV not infected status for the prospective population was 97.48% (1081 of 1109 patients). There were no differences among the presumptive diagnosis and risk groups for HCV infection in the percent positive or percent negative agreements.

Seroconversion Panels

Commercially available HCV patient seroconversion panels were tested using the ADVIA Centaur HCV assay to determine the seroconversion sensitivity of the assay. The performance of the ADVIA Centaur HCV assay on the seroconversion panels closely matched the performance of the reference assay. The following results were obtained:

Bayer ADVIA Centaur® HCV
Days to Evidence of HCV Infection
Seroconversion Panels

	Reference anti-HCV assay[a]		Centaur® HCV Assay[b]			Difference in Days to Anti-HCV Reactive Results[c]
Panel ID	N[d]	R[e]	N[d]	E[f]	R[e]	Reference – Centaur®
SC0400	11	14	11		14	0
SC0406	0	9	0		9	0
PHV905	11	14	7		11	3
6211	171	182	171		182	0
6213	37	43	35		37	6
6215	10	20	10		20	0
6222	36	40	36		40	0
6216	17	23	17		23	0
6226	37	39	32		37	2
6229	10	17	10		17	0
9058	7	10	7		10	0
6228	24	28	28		31	-3
9041	31	62	31		62	0
6227	46	74	46		74	0
9054	77	82	77		82	0
9047	21	28	21		28	0
SC0403	0	6	0		6	0
PHV908	13	19	5	11	13	6
6212	12	14	0		12	2
6214	25	30	25		30	0

a Reference HCV assay interpreted results: Reactive (R), or Nonreactive (N)
b ADVIA Centaur® HCV interpreted results: Reactive (R), Equivocal (E), or Nonreactive (N)
c The dates of the first reactive test results were compared in the reference assay and ADVIA Centaur® assay: if the first reactive test result occurred on the same day then difference = 0, if Centaur® had an earlier date then the difference was positive, otherwise negative.
d Post bleed day of last nonreactive result, usually denoted previous bleed from first reactive result.
e Post bleed day of first reactive result.
f Post bleed day of first equivocal result.
Note: Bleed day is calculated as the blood draw date for the appropriate result minus the date of first blood draw for the panel. The first draw date was bleed day 0.

Compared to the reference assay results, the first reactive time point for the ADVIA Centaur® HCV assay occurred earlier in 5 panels, at the same time in 14 panels, and later in 1 panel. Overall, compared to the reference anti-HCV assay, the ADVIA Centaur® HCV assay demonstrated efficacy for the detection of the appearance of anti-HCV following HCV infection.

Genotype Detection

Genotype detection was assessed using the Teragenix Corporation Genotype Panel. The Panel consisted of 25 human plasma samples that were predetermined by the supplier to include the most common recognized genotypes of HCV and their subtypes (1a, 1b, 1a/b, 2a/c, 3a, 4a, 4c/d, 4h). All of the anti-HCV positive panel members were observed to be reactive in the ADVIA Centaur® HCV assay. The ability of the ADVIA Centaur® HCV assay to detect antibodies to various HCV genotypes were also assessed by testing 100 individual genotype samples. These genotype samples included 20 type 1, 20 type 2, 20 type 3, 20 type 4, 10 type 4 non-A, and 10 type 5 samples. All of the confirmed HCV positive samples were found to be reactive in the ADVIA Centaur® HCV assay.

Potentially Cross -Reacting Subgroups

Samples from patients in the prospective population who were determined to be HBV infected or HAV infected were tested in the ADVIA Centaur® HCV assay and reference anti-HCV assay. Hepatitis B infection was determined to be acute, chronic, early recovery, recovery, or recovered stages of infection by HBsAg, HBeAg, anti-HBc Total, anti-HBc IgM, anti-HBeAg, and anti-HBs assays. Hepatitis A infection was determined from the medical history of the patient. Results of the anti-HCV assays were presented for patients with HCV infected status, HCV not determined status, and HCV not infected status by presumptive diagnosis and risk groups for HCV infection in the following table.

Bayer ADVIA Centaur® HCV
HCV Status and ADVIA Centaur® HCV Assay Results Among HBV Infected Patients as Determined by Marker Testing[a]
ADVIA Centaur® HCV Assay vs. HCV Status
All Testing Sites

	HCV Status[b]									
	Infected			*Not Determined*			*Not Infected*			
	ADVIA Centaur® HCV Assay[c]			*ADVIA Centaur® HCV Assay*			*ADVIA Centaur® HCV Assay*			
Presumptive Diagnosis and Risk Groups	*Reactive* N	*Equivocal* N	*Non-reactive* N	*Reactive* N	*Equivocal* N	*Non-reactive* N	*Reactive* N	*Equivocal* N	*Non-reactive* N	*Total[d]*
Signs and Symptoms	161	0	0	3	0	0	1	0	99	264
Hemophiliac	1	0	0	0	0	0	0	0	0	1
Intravenous drug user, current or past	65	0	0	0	0	0	1	0	2	68
Dialysis	5	0	0	1	0	0	0	0	20	26
Transfusion/Transplant	6	0	0	0	0	0	0	2	19	27
High Risk Sex[e]	7	0	0	3	0	0	0	0	35	45
Healthcare Worker	1	0	0	0	0	0	0	0	10	11
HIV Infected	0	0	0	0	0	0	0	0	4	4
Other[f]	1	0	0	0	0	0	0	0	19	20
None Specified	0	0	0	0	0	0	0	0	0	0
Overall	247	0	0	7	0	0	2	2	208	466

a Hepatitis B infected patients included acute, chronic, early recovery, recovery, and recovered stages of infection.
b Final HCV status was based on the reference test results and Chiron® RIBA® HCV 3.0 SIA supplemental testing of samples that were repeatedly reactive by reference anti-HCV assay testing.
c Final ADVIA Centaur® HCV was based on the initial test result and retest of initially reactive samples.
d Total number of test results by risk population.
e The high risk sex group includes patients with a diagnosis of a sexually transmitted disease, a sexual partner with a history of hepatitis, same sex sexual preference, multiple sex partners, HIV infected partner, or prostitutes.
f The Other risk group includes patients with the following risk factors: sharing straw cocaine, tattoo, history of incarceration, body piercing, family history of hepatitis, immunocompromised patient, tattoo artist, mortician or other known hepatitis exposure event.

Among patients in the prospective population who had ongoing or previous HBV infection (466 patients), the overall positive percent agreement between the ADVIA Centaur® HCV method and HCV infected status was 100.00% (247 of 247 HCV infected patients). The overall negative percent agreement between the ADVIA Centaur® HCV assay and HCV not infected status was 98.11% (208 of 212 HCV not infected patients) among patients in the prospective population who had ongoing or previous HBV infection.

Bayer ADVIA Centaur® HCV
HCV Status and ADVIA Centaur® HCV Assay Results Among HAV Infected Patients[a]
ADVIA Centaur® HCV Assay vs. HCV Status
All Testing Sites

Presumptive Diagnosis and Risk Groups	HCV Status[b]									
	Infected			Not Determined			Not Infected			
	ADVIA Centaur®-HCV Assay[c]			ADVIA Centaur® HCV Assay			ADVIA Centaur® HCV Assay			
	Reactive	Equivocal	Non-reactive	Reactive	Equivocal	Non-reactive	Reactive	Equivocal	Non-reactive	Total[d]
	N	N	N	N	N	N	N	N	N	
Signs and Symptoms	26	0	0	1	0	0	0	0	19	46
Hemophiliac	0	0	0	0	0	0	0	0	0	0
Intravenous drug user, current or past	2	0	0	0	0	0	0	0	2	4
Dialysis	0	0	0	0	0	0	0	0	2	2
Transfusion/Transplant	0	0	0	0	0	0	0	0	4	4
High Risk Sex[e]	2	0	0	0	0	0	0	0	7	9
Healthcare Worker	0	0	0	0	0	0	0	0	9	9
HIV infected	0	0	0	0	0	0	0	0	0	0
Other[f]	0	0	0	0	0	0	0	0	0	0
None Specified	0	0	0	0	0	0	0	0	0	0
Overall	30	0	0	1	0	0	0	0	43	74

a Patients with hepatitis type A.
b Final HCV status was based on the reference test results and Chiron® RIBA® HCV 3.0 SIA supplemental testing of samples that were repeatedly reactive by reference anti-HCV assay testing.
c Final ADVIA Centaur® HCV was based on the initial test result and retest of initially reactive samples.
d Total number of test results by risk population.
e The high risk sex group includes patients with a diagnosis of a sexually transmitted disease, a sexual partner with a history of hepatitis, same sex sexual preference, multiple sex partners, HIV infected partner, or prostitutes.
f The Other risk group includes patients with the following risk factors: sharing straw cocaine, tattoo, history of incarceration, body piercing, family history of hepatitis, immunocompromised patient, tattoo artist, mortician or other known hepatitis exposure event.

Among patients in the prospective population who had ongoing or previous HAV infection (74 patients), the overall positive percent agreement between the ADVIA Centaur® HCV method and HCV infected status was 100.00% (30 of 30 HCV infected patients), and the overall negative percent agreement with HCV not infected status was 100.00% (43 of 43 HCV not infected patients).

System Reproducibility

The ADVIA Centaur® HCV precision and reproducibility study was performed at 3 external sites utilizing 2 reagent lots per site. Three reagent lots were used for the study. A 5-member panel and controls were assayed in replicates of 5 on a single run per day over 6 days for each lot. The study was completed with a single calibration of the assay (one calibration interval). Standard deviation and percent CV were calculated for within run, between run, and total. The data from all 3 sites and from all 3 reagent lots were combined to achieve SD and percent CV for within run, between run, between testing site, between lot, and total. The precision estimates were derived from variance component analysis. The reproducibility results are presented in the following table.

Bayer ADVIA Centaur® HCV Assay
Reproducibility
Between Testing Sites and Between Reagent Lots Estimates
(Across All Reagent Lots and All Testing Sites)

Panel Member	Mean ADVIA Centaur® HCV Index Value	Within Run[a]		Between Run[b]		Between Testing Site[c]		Between Lot[d]		Total[e]		Number of Observations
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
1	0.03	0.004	NA	0.005	NA	0.041	NA	0.011	NA	0.043	NA	180
2	1.22	0.066	5.43	0.076	6.23	0.000	0.00	0.076	6.21	0.126	10.34	180
3	3.95	0.177	4.48	0.288	7.29	0.070	1.77	0.363	9.19	0.501	12.68	180
4	6.32	0.267	4.22	0.376	5.95	0.221	3.49	0.718	11.36	0.881	13.94	180
5	9.56	0.716	7.49	0.553	5.78	0.494	5.16	0.855	8.94	1.339	14.00	180
Negative Control	0.08	0.014	NA	0.012	NA	0.032	NA	0.057	NA	0.068	NA	180
Positive Control	5.72	0.404	7.06	0.328	5.73	0.387	6.76	0.406	7.09	0.765	13.36	180

a Variability of the assay performance within day (all testing sites and reagent lots).
b Variability of the assay performance between days (all testing sites and reagent lots).
c Variability of the assay performance between testing sites (from testing site to testing site).
d Variability of the assay performance between reagent lots (from reagent lot to reagent lot, across all testing sites).
e Variability of the assay performance incorporating all testing sites, all reagent lots, and all days.
NA = Not applicable
Note: 5 replicates per panel in 1 run per day for 6 days

Cross-Reactivity

The ADVIA Centaur HCV assay was evaluated for potential cross-reactivity with other viral infections and disease state specimens. The reactive HCV status of each specimen was verified using an anti-HCV reference assay. The following results were obtained using the ADVIA Centaur HCV assay:

		Number of Reactive Anti-HCV Results	
Clinical Category	Number Tested	ADVIA Centaur Assay	Reference Assay
Hepatitis A Infection (HAV)	5	0	0
Non-viral Liver Disease	10	1	1
Epstein-Barr Virus (EBV) IgG	10	0	0
Epstein-Barr Virus (EBV) IgM	10	0	0
Herpes Simplex Virus (HSV) IgG	10	0	0
Herpes Simplex Virus (HSV) IgM	10	0	0
Syphilis IgG	14	0	0
Human Immunodeficiency Virus (HIV1/2)	10	1	1
Varicella Zoster (VZV) IgG	10	0	0
Cytomegalovirus (CMV) IgG	10	0	0
Cytomegalovirus (CMV) IgM	3	0	0
Rubella IgG	10	0	0
Toxoplasma IgG	10	0	0
Multiparity	10	0	0
Flu Vaccine Recipient	10	2	2
Rheumatoid Arthritis (RF)	9	1	1
Anti-Nuclear Antibody (ANA) & Systemic Lupus Erythematosus (SLE)	7	0	0
Total Samples Tested	1158	5	5

Endogenous Interferents

The ADVIA Centaur HCV assay was evaluated for interference according to NCCLS Document EP7-P[13]. None of the interferents at the levels tested produced a change in clinical interpretation of the assay.

Serum specimens that are...	Demonstrate ≤10% change in results up to...
hemolyzed	500 mg/dL of hemoglobin
lipemic	1000 mg/dL of triglycerides
icteric	60 mg/dL of conjugated bilirubin
icteric	40 mg/dL of unconjugated bilirubin
proteinemic	12 g/dL of protein
proteinemic	3.5 g/dL of protein

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

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Manufactured for Ortho-Clinical Diagnostics, Inc. by Bayer HealthCare LLC, Tarrytown NY

Ortho-Clinical Diagnostics
a Johnson & Johnson company

Anti-HCV Reagent Pack

PIGEM1240/101.0

EN
FR
DE
IT
PT
ES
DA
SV
EL

Symbols used • Symboles utilisés • Symbol-Legende • Simboli utilizzati • Simbologia • Símbolos empleados • Anvendte symboler • Symboler som används • Σύμβολα που χρησιμοποιούνται

Use by/Expiration date (Year–Month–Day) • Utiliser jusque/Date d'échéance (Année- mois-jour) • Verfallsdatum (Jahr-Monat-Tag) • Utilizzare entro/Data di scadenza (Anno-Mese-Giorno) • Prazo de validade (ano-mês-dia) • Fecha de caducidad (año-mes-día) • Anvendes før/Udløbsdato (År-Måned-Dato) • Använd före/Utgångsdatum (år-månad-dag) • Χρήση έως/Ημερομηνία λήξης (Έτος-Μήνας-Ημέρα)

LOT — Lot Number • Numéro de lot • Losnummer • Numero di lotto • Número de lote • Número de lote • Lotnummer • Lot-nummer • Αριθμός παρτίδας

SN — Serial number • Numéro de série • Seriennummer • Numero di serie • Número de série • Número de serie • Serienummer • Serienummer • Σειριακός αριθμός

REF — Catalogue number • Référence du catalogue • Artikelnummer oder Produktcode • Numero di catalogo • Número de catálogo • Código de producto • Katalognummer • Katalognummer • Αριθμός καταλόγου

Attention, see instructions for use • Attention, voir la notice d'utilisation • Achtung, siehe Gebrauchsanweisung • Attenzione, vedere le istruzioni per l'uso • Atenção, verificar as instruções de utilização • Atención, ver las instrucciones de uso • Obs., se brugsanvisning • Obs! Se bruksanvisningen • Προσοχή, βλ. οδηγίες χρήσης

Manufacturer • Fabricant • Hersteller • Produttore • Fabricante • Fabricante • Producent • Tillverkare • Παρασκευαστής

EC REP — Authorized representative • Mandataire • Bevollmächtigter in der EU • Rappresentante autorizzato • Representante autorizado • Representante autorizado • Autoriseret repræsentant • Auktoriserad representant • Εξουσιοδοτημένος αντιπρόσωπος

Σ — Contains sufficient tests for 'n' tests • Quantité suffisante pour 'n' tests • Inhalt ausreichen für 'n' Tests • Contiene test sufficienti per 'n' test • Contém o suficiente para "n" testes • Contiene un número suficiente para "n" tests • Indeholder tilstrækkelige test til "n" test • Innehåller tillräcklig mängd för 'n' analyser • Περιέχει επαρκή ποσότητα για 'n' εξετάσεις

Σ = 'n' tests

IVD — *In vitro* diagnostic medical device • Pour diagnostic *in vitro* • *In-vitro*-Diagnostikum • Dispositivo medico diagnostico *in vitro* • Para diagnóstico *in vitro* • Producto sanitario para diagnóstico *in vitro* • Medicinsk udstyr til *in vitro*-diagnostik • Medicinsk anordning för *in vitro*-diagnostik • *In vitro* διαγνωστική ιατρική συσκευή

Temperature limitation/Store between • Limites de température/Conserver à • Temperaturbeschränkung/Lagerung bei • Limiti di temperatura/Conservare ad una temperatura compresa tra • Conservar a uma temperatura entre • Límite de temperatura/Conservar a una temperatura de • Temperaturbegrænsning/Opbevares mellem • Temperaturgräns/Förvara mellan • Περιορισμός θερμοκρασίας/Διατηρήστε σε θερμοκρασία μεταξύ

Consult instructions for use • Consultez la notice d'utilisation • Siehe Gebrauchsanweisung • Consultare le istruzioni per l'uso • Consultar as instruções de utilização • Atención, ver las instrucciones de uso • Se brugsanvisningen • Se bruksanvisningen • Συμβουλευτείτε τις οδηγίες χρήσης

EN

Vitros Immunodiagnostic Products
Anti-HCV Reagent Pack

PIGEM1240/101.0

Intended Use

For the *in vitro* qualitative detection of antibodies to hepatitis C virus (anti-HCV) in human serum and plasma (EDTA, heparin or citrate).

Summary and Explanation of the Test

The hepatitis C virus (HCV) is now known to be the causative agent for most, if not all, blood-borne non-A, non-B hepatitis (NANBH). Studies throughout the world indicate that HCV is transmitted through contaminated blood and blood products, through blood transfusions or through other close, personal contacts. The presence of anti-HCV indicates that an individual may have been infected with HCV and may be capable of transmitting HCV infection[1].

Three recombinant hepatitis C virus encoded antigens are used in the *Vitros* Anti-HCV assay. The three recombinant antigens are c22-3, c200 and NS-5. The recombinant protein c22-3 is encoded by the putative core region of the HCV genome. HCV recombinant protein c200 is encoded by the putative NS3 and NS4 regions of the HCV genome. The c200 protein contains the c33c protein sequence which is genetically linked to the c100-3 protein sequence. Studies have indicated that antibodies which develop after infection with HCV are often reactive with c22-3 and or c33c[2]. HCV recombinant protein NS5 is encoded by the putitive NS5 region of the HCV genome. A significant proportion of persons infected with HCV develop antibodies to NS5[3].

The host organism for all three HCV recombinant antigens is *S. cerevisiae* (yeast).

Principles of the Procedure

The *Vitros* Anti-HCV assay is performed using the *Vitros* Anti-HCV Reagent Pack and the *Vitros* Immunodiagnostic Products Anti-HCV Calibrator on the *Vitros* Immunodiagnostic System.

An immunometric technique is used, this involves a two stage reaction. In the first stage HCV antibody present in the sample binds with HCV recombinant antigens coated on the wells. Unbound sample is removed by washing. In the second stage horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-human IgG) binds to any human IgG captured on the well in the first stage. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction[4]. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the *Vitros* System. The amount of HRP conjugate bound is directly proportional to the level of anti-HCV present.

Warnings and Precautions

For *In Vitro* Diagnostic Use Only

Warning - Potentially Infectious Material

- Treat as if capable of transmitting infection.
- Handling of samples and assay components, their use, storage, and solid and liquid waste disposal should be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation (e.g. NCCLS Guideline M29[5]).

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The *Vitros* Anti-HCV Calibrator contains:
HCV antibody positive plasma obtained from donors who were tested individually and who were found to be negative for hepatitis B surface antigen, and for antibodies to the human immunodeficiency virus (HIV 1+ 2), using approved methods (enzyme immunoassays). The HCV antibody positive plasma has been treated in order to reduce the titer of potentially infectious virus. However, as no testing method can rule out the risk of potential infection, handle as if capable of transmitting infection.

HCV antibody negative plasma obtained from donors who were tested individually and who were found to be negative for hepatitis B surface antigen, and for antibodies to HCV and HIV 1+ 2, using approved methods (enzyme immunoassays).

Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+ 2 or other infectious agents are absent.

Warning - Contains ProClin 300 and 2-Chloroacetamide
The Conjugate Reagent contains ProClin 300 and the Assay Reagent contains 2-Chloroacetamide. R43: May cause sensitisation by skin contact. R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. S24: Avoid contact with skin. S37: Wear suitable gloves.

Materials Provided

1 reagent pack containing:
- 100 coated wells (Hepatitis C Virus recombinant antigens derived from yeast; coated at 0.41 µg/well).
- 18.2 mL Assay Reagent - (buffer with anti-microbial agent).
- 20.6 mL Conjugate Reagent - (HRP-mouse monoclonal anti-human IgG, 1.04 ng/well) in buffered foetal calf serum with anti-microbial agent.

Note: Contains bovine serum albumin and foetal calf serum.

Materials Required but not Provided

Vitros Immunodiagnostic System and the following *Vitros* Immunodiagnostic Products: Anti-HCV Calibrator (including lot calibration card and protocol card), Signal Reagent, Universal Wash Reagent, Reagent Pack Storage Box (optional) with desiccant.

Reagent Preparation and Storage

The reagent pack is supplied ready for use. Store unopened at 2-8 °C, do not freeze. Use opened reagent packs within 8 weeks of first loading onto a System; do not use beyond the expiration date. Store opened reagent packs on board the System, or at 2-8 °C in a sealed reagent pack storage box containing dry desiccant.

The Anti-HCV Calibrator is supplied ready for use. Store unopened at 2-8 °C. Do not use beyond the expiration date. After opening store for up to 13 weeks at 2-8 °C or 13 weeks at -20 °C (with no more than 1 freeze-thaw cycle).

Patient Preparation

No special patient preparation is necessary.

Sample Collection, Preparation and Storage

Serum or plasma (EDTA, heparin or citrate) samples may be used. Results from citrate plasma samples will be proportionally lower due to dilution by the liquid anti-coagulant. Collect blood samples using standard procedures. Samples should be thoroughly separated from all cellular material. Failure to do so may lead to a falsely elevated result. Serum and plasma samples may be stored for up to 7 days at 2-8 °C or 4 weeks at -20 °C. Avoid repeated freezing and thawing.

Sample collection devices have on occasion been reported to be detrimental to the integrity of certain analytes and could interfere with some method technologies[4]. Owing to the variety of sample collection devices available, Ortho-Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. It is recommended that each user ensures that the chosen device is used according to the manufacturer's instructions and is compatible with this *Vitros* assay.

Quality Control and Procedural Notes

- Handle the reagent pack with care, avoid the following: allowing condensation to form on the pack; causing reagents to foam; agitation of the pack.
- Calibration is lot specific; reagent packs and calibrators are linked by lot number. Reagent packs from the same lot may use the same calibration, which must be performed using a calibrator of the same lot number.

- Thoroughly mix samples, calibrators and controls by inversion and bring to 15-30 °C before use.
- Handle samples, calibrators and controls in stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time samples, calibrators and controls are on board the *Vitros* System. Refer to the *Vitros* System Operator's Guide for further information. Return to 2-8 °C as soon as possible after use, or load only sufficient for a single use. The calibrator may be aliquoted into alternative containers, which may be bar coded with the labels provided.
- The Anti-HCV Calibrator is automatically processed in duplicate.
- Check the inventory regularly to aid the management of reagents and ensure that sufficient *Vitros* Signal Reagent, *Vitros* Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of assays on a single sample, ensure that the sample volume is sufficient for the assays ordered.
- Good laboratory practice requires that controls be processed to verify the performance of the assay. There are 2 *Vitros* Anti-HCV Controls (Anti-HCV negative and Anti-HCV positive). It is recommended that controls are processed when a calibration is performed, and subsequently at least once every 24 hours and after specified service procedures are performed (Refer to the *Vitros* System Operator's Guide). If quality control procedures within your laboratory require more frequent use of controls, follow those procedures. For more detailed information, refer to the *Vitros* System Operator's Guide.
- The default assay name which will appear on patient reports is Anti-HCV. The default short name that will appear on the assay selection menus and laboratory reports is aHCV. These defaults may be reconfigured, if required, in the Options & Configurations - Configure Analytes screen.

Procedure

The *Vitros* Anti-HCV assay requires 20 µL sample, calibrator or control for a singleton determination. This does not take account of the minimum fill volume of the chosen sample container.

The *Vitros* Anti-HCV assay must be calibrated each time a new reagent lot is used, and subsequently at intervals of 28 days. The *Vitros* Anti-HCV assay may also need to be calibrated after certain service procedures are performed or if quality control results are consistently outside your acceptable range.

For detailed instructions on the operation of the System refer to the *Vitros* Immunodiagnostic System Operator's Guide, Chapters 4-7. In summary:

1. Scan the protocol card to load a new assay protocol onto the System. The assay button is then displayed on the Sample Programming screen. Scan the lot calibration card for each new reagent lot to enter lot specific calibration and expiration information.
2. Open the foil pouch and remove the reagent pack. Load the pack at the auto-load station, or use the Unload/Load button on the Reagent Management - View by Reagent screen. **Note:** Do not use damaged or incompletely sealed product.
3. Load samples into universal sample trays, using adapters where necessary (samples may be bar coded if desired). Place a disposable tip adjacent to each sample and load the trays onto the System. Define sample programs using the Sample Programming screen. Start the sampling operation, all sample processing steps will be carried out automatically.
4. Process the calibrator in the same manner as samples (loading sufficient for the automatic duplicate determination). Calibration need not be programmed if bar code labels are used; calibration will be initiated automatically.

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Results

Results are calculated as a normalised signal, relative to a cut-off value. During the calibration process a lot-specific parameter, encoded on the lot calibration card, is used to determine a valid stored cut-off value for the System. For further details on calibration refer to the instructions for use supplied with the Anti-HCV Calibrator. Results are automatically calculated by the *Vitros* System.

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cut-off value}}$$

A result of ≥1.00 indicates a reactive sample and the possible presence of Anti-HCV.
A result <0.90 indicates a non-reactive sample, negative for Anti-HCV.
A result of ≥0.90 and <1.00 indicates a borderline sample.

Quality Control

- Calibration results are assessed against two quality parameters as detailed in View Cal Parameters, accessed via the Options & Configuration - Review/User Calibrations screen. Failure to meet either of the defined quality parameter ranges will be coded in the calibration report. For actions to be taken following a failed calibration refer to the Operator's Guide, Chapter 8, Reviewing Results.
- Patient sample values will be flagged 'Negative', 'Borderline' or 'Reactive'. Control values will be flagged when ≥2 SDs from the defined baseline mean.
- The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with control values to determine the validity of the calibration.
- If control results fall outside of your acceptable range, investigate the cause before deciding whether to report patient results.

Limitations of the Procedure

- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to or infection with HCV. HCV antibodies may be undetectable in some stages of the infection and in some clinical conditions[20].
- Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays[18]. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results which are inconsistent with clinical observations indicate the need for additional testing.
- Samples containing triolein (<33.9 mmol/L), hemoglobin (<5 g/L) or bilirubin (<0.342 mmol/L) do not interfere with the clinical interpretation of these results. Do not use turbid samples.

Interpretation of Results

A sample found borderline or reactive must be retested in duplicate to verify its status. Before retesting, the sample should be centrifuged to ensure freedom from cells, cellular debris or fibrin. If results on repeat testing are <0.90 for both replicates, the sample should be considered negative. If either duplicate retest result is ≥0.90, the sample should be tested by supplemental tests to confirm the result. A repeatedly reactive sample, confirmed by supplemental tests should be considered positive for anti-HCV. In the case of repeatedly borderline results, analysis of follow-up samples is recommended.

Performance Characteristics

Calibration and Traceability of Calibration

The calibration of the *Vitros* Anti-HCV assay is traceable to an in-house reference calibrator which has been value assigned to optimize the clinical sensitivity and specificity performance.

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Precision
Precision was evaluated according to the National Committee for Clinical Laboratory Standards protocol EP5-T2[9]. Two replicates of each of 4 panel samples were assayed on a single occasion per day on at least 20 different days. The experiment was performed using 2 reagent lots on different Systems. The data presented are representative of the product performance.

Table 1: Precision

Representative Result	Within-run SD*	Within-run CV(%)*	Within-calibration SD*	Within-calibration CV(%)*	Within-lab SD*	Within-lab CV(%)*
0.14	0.00336	2.4	0.00835	4.9	0.00945	6.9
2.04	0.044	2.1	0.132	6.4	0.134	6.6
5.74	0.132	2.4	0.339	5.8	0.354	6.2
14.9	0.187	1.2	0.454	3.1	0.576	3.9

* Root Mean Square (RMS)

Within-run: Between-duplicate precision averaged over all runs.
Within-calibration: Total precision, with weighted components of within-run and between-day variation.
Within-lab: A measure of the effect of recalibration on total precision, calculated within reagent lot, using data from 4 calibrations.

Sensitivity
435 patient samples previously determined as positive by a HCV immunoblot assay were tested in the *Vitros* Anti-HCV assay. The sensitivity for this population of samples in the *Vitros* Anti-HCV assay was calculated as 100% (435/435).

In addition, 29 commercially available seroconversion panels were tested. The *Vitros* Anti-HCV assay showed equivalent or greater seroconversion sensitivity for 29/29 panels, when compared to the published results from another commercially available assay.

Specificity
Samples from 5 374 presumed healthy blood donors, and 393 clinical specimens were tested in the *Vitros* Anti-HCV assay and another commercially available assay.

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Samples	Number of test samples	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Donor	5 374	14	13	0
Clinical	393	1	1	0

The specificity for the *Vitros* Anti-HCV assay for the donor population was calculated as 99.76% (5 361/5 374) based on repeat reactives. The specificity for the *Vitros* Anti-HCV assay for the clinical population was calculated as 99.75% (392/393) based on repeat reactives.

Additionally 161 samples from the following potentially cross-reacting sub-groups were tested in the *Vitros* Anti-HCV assay: CMV positive, EBV positive, HIV positive, non-viral liver disease patients, other viral liver diseases (eg HBV, HAV), SLE, rheumatoid factor positive, recent vaccinees (eg flu), yeast reactive samples. Of these categories none were found to result in false reactives in the *Vitros* Anti-HCV assay.

ADM working group: Summary of Evaluation

The *Vitros* Anti-HCV assay was evaluated by the ADM working group. 450 samples from 246 patients infected with different genotypes of Hepatitis C virus and 50 samples from the HCV SFTS panel, including 27 RNA reactive samples, were tested. The *Vitros* Anti-HCV assay showed good sensitivity for the detection of early seroconversion and for the screening of other categories of reactive samples, including those showing an isolated reactivity and those from chronic carriers.

A population of 2018 blood donor samples were tested with the *Vitros* Anti-HCV assay. Eight samples gave repeatably reactive results, of which 1 was confirmed as reactive and 6 were found negative by other assays. The result of the remaining sample could not be interpreted (indeterminate NS3, PCR negative). The specificity for the *Vitros* Anti-HCV assay was approximately 99.70%.

Licence Statement

HCV recombinant antigens used in the *Vitros* Anti-HCV assay are prepared under U.S. licence by Chiron Corporation under a shared manufacturing agreement.

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Vitros Immunodiagnostic Products
Cartouche de réactifs anti-VHC

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Application

A utiliser *in vitro* pour la détection qualitative des anticorps dirigés contre le virus de l'hépatite C (anticorps anti-VHC) dans le sérum et le plasma humains (EDTA, héparine ou citrate).

Résumé et principe du test

Le virus de l'hépatite C (VHC) est reconnu comme étant responsable de la plupart, si ce n'est de toutes, les hépatites non A-non B transmissibles par voie sanguine. Des études à l'échelle mondiale ont montré que le VHC se transmet par le sang ou les produits sanguins contaminés, par transfusions sanguines ou par contacts physiques avec l'entourage du patient. La détection d'anticorps anti-VHC chez un individu indique que ce dernier peut avoir été infecté par le VHC et qu'il est donc potentiellement contaminant[1].

Le test anti-VHC *Vitros* utilise trois antigènes recombinants spécifiques du virus de l'hépatite C, à savoir les antigènes c22-3, c200 et NS-5. La protéine recombinante c22-3 est codée par la région présumée du core du génome du VHC. La protéine recombinante c200 est codée pour les régions présumées NS3 et NS4 du génome du VHC. La protéine c200 contient la séquence de la protéine c33c qui est génétiquement liée à la séquence de la protéine c100-3. Des études ont mis en évidence que les anticorps développés après une infection par le VHC réagissent souvent avec les protéines c22-3 et/ou c33c[2]. La protéine recombinante NS5 est codée par la région présumée NS5 du génome du VHC. Une proportion significative de patients infectés par le VHC développent des anticorps anti-NS5[3].

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L'organisme hôte des trois antigènes recombinants pour le VHC est la *S. cerevisiae* (levure).

Principe du test

Le test *Vitros* Anti-VHC est réalisé à l'aide d'une cartouche de réactifs *Vitros* Anti-VHC et de l'étalon *Vitros* Anti-VHC sur le Système d'immunodiagnostic *Vitros*.

Ce test repose sur une technique immunométrique impliquant une réaction en deux étapes. Dans un premier temps, l'anticorps anti-VHC présent dans l'échantillon se lie aux antigènes recombinants du VHC qui revêtent les parois du puits. L'échantillon non lié est éliminé par lavage. Dans un second temps, un conjugué d'anticorps (anticorps monoclonal de souris anti-IgG humaine) marqué à la peroxydase de raifort (HRP) se fixe à toute IgG d'origine humaine capturée sur les parois du puits lors de la première étape. Le conjugué non lié est éliminé par lavage.

Le conjugué HRP lié est mesuré par une réaction luminescente[4]. Un réactif contenant des substrats luminogènes (un dérivé du luminol et un sel peracide) et un agent de transfert des électrons est ajouté dans les puits. Le HRP du conjugué lié catalyse l'oxydation du dérivé du luminol, produisant de la lumière. L'agent de transfert des électrons (un acétalinide substitué) augmente l'intensité de la lumière produite et prolonge son émission. Les signaux lumineux sont lus par le Système *Vitros*. La quantité de conjugué lié est directement proportionnelle à la quantité d'anticorps anti-VHC présents dans l'échantillon.

Avertissements et précautions

Pour le diagnostic *in vitro* uniquement

Attention : Produits potentiellement infectieux

- Manipuler comme s'il s'agissait d'un produit potentiellement infectieux.
- La manipulation des échantillons et composants du test, leur utilisation, leur stockage et l'élimination de leurs déchets solides ou liquides doivent être en accord avec les procédures définies par les réglementations nationales en vigueur (par exemple NCCLS Guideline M29[5]).

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L'étalon Anti-VHC *Vitros* contient :
Du plasma positif pour les anticorps anti-VHC provenant de donneurs ayant subi des tests individuels et chez lesquels le dépistage par des méthodes agrées (dosages immuno-enzymatiques) de l'antigène de surface de l'hépatite B et des anticorps dirigés contre le virus de l'immunodéficience humaine (VIH 1+ 2) s'est avéré négatif. Le plasma positif pour les anticorps anti-VHC a été traité afin de réduire le titre du virus potentiellement infectieux. Cependant, aucune méthode de test ne pouvant éliminer le risque d'une infection potentielle, le manipuler comme s'il était contaminant.

Du plasma négatif pour les anticorps anti-VHC provenant de donneurs ayant subi des tests individuels et chez lesquels le dépistage par des méthodes agrées (dosages immuno-enzymatiques) de l'antigène de surface de l'hépatite B, et des anticorps dirigés contre le VHC et le HIV 1+2 s'est avéré négatif.

Toutes les précautions nécessaires doivent être prises lors de la manipulation de produits d'origine humaine. Tout échantillon doit être considéré comme potentiellement infectieux. Aucune méthode de dépistage ne peut totalement garantir l'absence du virus de l'hépatite B, du VHC, du VIH 1+2 ou de tout autre agent infectieux.

Attention – Contient du ProClin 300 et 2-Chloroacétamide

Le réactif conjugué contient du ProClin 300 et le réactif de dosage contient 2-chloroacétamide. R43 : Peut entraîner une sensibilisation par contact avec la peau. R52/53 : Nocif pour les organismes aquatiques, peut entraîner des effets néfastes à long terme pour l'environnement aquatique. S24 : Éviter le contact avec la peau. S37 : Porter des gants appropriés.

Matériel fourni

1 cartouche de réactifs contenant :
- 100 puits revêtus (antigènes recombinants du virus de l'hépatite C provenant de levure ; revêtus à 0,41 µg/puits).
- 18,2 mL de réactif de dosage (solution tampon avec agent antimicrobien).
- 20,6 mL de réactif conjugué (HRP-anticorps monoclonal de souris anti-IgG humaine, 1,04 ng/puits) en solution tampon de sérum fœtal de veau avec agent antimicrobien.

Note: Contient de la sérumalbumine bovine et du sérum fœtal de veau.

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Matériel nécessaire mais non fourni

Le Système d'immunodiagnostic *Vitros* et les produits d'immunodiagnostic *Vitros* suivants : étalon anti-VHC (y compris carte d'étalonnage de lot et carte de protocole), réactif signal, réactif de lavage universel et boîte de conservation de cartouches de réactifs (facultatif) avec dessiccant.

Préparation et conservation des réactifs

La cartouche de réactifs est fournie prête à l'emploi. Conserver les réactifs non ouverts entre 2-8 °C. Ne pas congeler. Utiliser les cartouches de réactifs ouvertes dans les 8 semaines suivant le premier chargement dans le Système ; ne pas utiliser au-delà de la date de péremption. Après ouverture, conserver les cartouches de réactifs à bord du Système ou entre 2 et 8 °C, dans un coffret de conservation de cartouches de réactifs hermétiquement fermé et contenant du déshydratant sec.

L'étalon anti-VHC est prêt à l'emploi. Il doit être conservé non ouvert entre 2-8 °C. Ne pas utiliser au-delà de la date de péremption. Après ouverture, conserver jusqu'à 13 semaines entre 2 et 8 °C ou 13 semaines à -20 °C (sans dépasser 1 cycle de congélation/décongélation).

Préparation du patient

Aucune préparation particulière du patient n'est nécessaire.

Prélèvement, préparation et conservation des échantillons

Des échantillons de sérum ou de plasma (EDTA, héparine ou citrate) peuvent être utilisés. Les résultats des échantillons plasmatiques citratés seront proportionnellement inférieurs en raison de leur dilution dans l'anticoagulant. Prélever les échantillons sanguins selon les procédures standard. Les échantillons doivent être soigneusement séparés de tout matériel cellulaire. Une séparation mal effectuée pourrait conduire à des résultats faussement élevés. Les échantillons de sérum et de plasma peuvent être conservés jusqu'à 7 jours entre 2 et 8 °C ou 4 semaines à -20°C. Eviter les congélations et décongélations répétées.

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Certains matériaux de prélèvement peuvent être néfastes à l'intégrité de certains analytes et peuvent interférer avec certaines techniques de dépistage[16]. Du fait de la variété des matériaux disponibles pour le prélèvement d'échantillons, Ortho-Clinical Diagnostics ne peut fournir de données définitives concernant la performance de chacun de ses produits en présence de ces matériaux. Il est recommandé à l'utilisateur de s'assurer que le matériel choisi est compatible avec ce test *Vitros* et est utilisé en conformité avec les instructions du fabricant.

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Contrôle de qualité et recommandations concernant la procédure

- Manipuler la cartouche de réactifs avec précaution : éviter la formation de condensation sur la cartouche ; éviter la formation de mousse ; éviter d'agiter la cartouche.
- A chaque étalonnage correspond un lot particulier de cartouches de réactifs et d'étalons qui portent le même numéro. Des cartouches de réactifs du même lot peuvent utiliser le même étalonnage, qui doit être effectué à l'aide d'étalon portant le même numéro de lot.
- Mélanger soigneusement les échantillons, l'étalon et les contrôles par inversion et les laisser revenir à température ambiante, entre 15-30 °C, avant utilisation.
- Manipuler les échantillons, les étalons et les contrôles dans des récipients bouchés pour éviter la contamination et l'évaporation. Pour éviter l'évaporation, limiter la durée pendant laquelle les échantillons, les étalons et les contrôles sont à bord du système *Vitros*. Pour plus d'informations, se reporter au Manuel de l'opérateur du système *Vitros*. Ramener dès que possible l'étalon à une température comprise entre 2 et 8 °C après utilisation, ou n'en charger dans le Système qu'une quantité suffisante pour une seule utilisation. L'étalon peut être aliquoté dans d'autres contenants sur lesquels on apposera les étiquettes à codes à barres prévues à cet effet.
- L'étalon anti-VHC est automatiquement traité en double.

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- Vérifier régulièrement les stocks de réactifs et s'assurer qu'une quantité suffisante de réactif signal *Vitros*, de solution de lavage universelle *Vitros* et de réactif étalonné est disponible pour le travail prévu. Lors de la réalisation d'une série de tests à partir d'un seul échantillon, s'assurer que le volume d'échantillon est suffisant pour les tests demandés.
- Conformément aux bonnes pratiques de laboratoire, des contrôles doivent être inclus dans le test afin d'en vérifier les performances. Il y a 2 contrôles Anti-VHC *Vitros* (Anti-VHC négatif et Anti-VHC positif). Il est recommandé de tester les contrôles lors de chaque étalonnage, puis au moins toutes les 24 heures et après l'exécution de certaines procédures de maintenance (se reporter au Manuel de l'opérateur du système de *Vitros*). Si les procédures de contrôle qualité de votre laboratoire exigent un recours plus fréquent aux contrôles, il convient d'appliquer ces procédures. Pour plus d'informations, se reporter au Manuel de l'opérateur.
- Le nom par défaut du test apparaissant sur les rapports patient est Anti-VHC. L'abréviation par défaut apparaissant sur les menus de sélection des tests et sur les rapports de laboratoire est aVHC. Ces noms par défaut peuvent être reconfigurés si nécessaire, dans l'écran Options & Configurations - Configuration des paramètres.

Protocole

Le test *Vitros* Anti-VHC nécessite 20 µL d'échantillon, d'étalon ou de contrôle pour une détermination en simple exemplaire. Cela ne tient pas compte du volume mort du contenant échantillon choisi.

Le test *Vitros* Anti-VHC doit être étalonné chaque fois qu'un nouveau lot de cartouche de réactifs est utilisé, et par la suite, tous les 28 jours. Le test *Vitros* Anti-VHC peut également nécessiter un étalonnage après que certaines procédures de maintenance ont été exécutées ou si les résultats des contrôles de qualité sont régulièrement en dehors de la gamme acceptable.

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Vous trouverez des instructions détaillées relatives à l'utilisation du Système dans le Manuel de l'Opérateur du Système Immunodiagnostic *Vitros* aux chapitres 4-7. En voici un résumé :

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1. Lire la carte de protocole pour charger un nouveau protocole de test dans le Système. La touche du test s'affiche alors à l'écran Programmation des échantillons. Lire la carte d'étalonnage de lot de chaque nouveau lot de réactifs pour entrer les informations spécifiques au lot relatives à l'étalonnage et à la date de péremption.
2. Ouvrir le sachet en aluminium et retirer la cartouche de réactifs. Charger cette cartouche dans la station de chargement automatique ou utiliser la touche Déchargement/Chargement de l'écran Gestion des réactifs - Afficher par réactif. **Note:** Les produits provenant de boîtes endommagées ou d'un emballage mal scellé ne doivent pas être utilisées.
3. Charger les échantillons dans les portoirs universels pour échantillon, en utilisant si nécessaire des adaptateurs (les échantillons peuvent être dotés d'un code à barres). Placer un embout jetable près de chaque échantillon et charger les portoirs dans le Système. Définir les programmes de traitement des échantillons par l'écran Programmation des échantillons. Lancer la procédure d'échantillonnage : toutes les étapes du traitement s'exécutent automatiquement.
4. Traiter l'étalon de la même manière que les échantillons (en chargeant une quantité suffisante pour un test en double automatique). Il n'est pas nécessaire de programmer l'étalonnage si des étiquettes à codes à barres sont utilisées, l'étalonnage démarre automatiquement.

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Résultats

Les résultats sont calculés sous forme d'un signal normalisé, par rapport à une valeur seuil. Lors de la procédure d'étalonnage, un paramètre propre au lot, codé sur la carte d'étalonnage de lot, est utilisé afin de déterminer une valeur seuil valide enregistrée pour le Système. Pour plus de détails sur l'étalonnage, se reporter à la notice fournie avec les étalons *Vitros* anticorps Anti-VHC. Les résultats sont calculés automatiquement par le système *Vitros*.

$$\text{Résultat} = \frac{\text{signal de l'échantillon test}}{\text{valeur seuil}}$$

Un résultat ≥1,00 témoigne d'un échantillon positif et de la présence possible d'anticorps anti-VHC.
Un résultat <0,90 témoigne d'un échantillon non réactif, négatif pour les anticorps anti-VHC.
Un résultat ≥0,90 et <1,00 témoigne d'un échantillon limite.

Contrôle de qualité

- Les résultats de l'étalonnage sont évalués par rapport à deux paramètres de qualité détaillés dans l'option Afficher param. d'étalonnage, accessible à partir de l'écran Options & Configuration - Afficher/Etalonnage utilisateurs. Toute absence de correspondance avec l'un des paramètres de qualité sera codée dans le rapport d'étalonnage. Pour plus d'informations sur les mesures à prendre en cas d'échec de l'étalonnage, se reporter au Guide de l'opérateur, Chapitre 8, Visualisation des résultats.
- Les résultats des échantillons patient porteront les indicateurs 'Négatif', 'Limite' ou 'Réactif'. Les valeurs de contrôle sont signalées par un indicateur dès qu'elles sont ≥2 écarts types par rapport à la moyenne.
- La qualité de l'étalonnage ne peut être déterminée à partir d'un seul paramètre : sa validité doit être établie à l'aide du rapport d'étalonnage, utilisé conjointement avec des valeurs de contrôle.
- Si les résultats des contrôles sortent des limites acceptables, en étudier la cause avant de décider de rapporter ou non les résultats patients.

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Limites de validité du test

- Les résultats obtenus à partir de cette trousse ou de n'importe quelle autre trousse de diagnostic ne doivent être exploités et interprétés que dans le contexte d'un profil clinique complet. Un résultat de test négatif n'exclut pas la possibilité d'une exposition ou infection au/par VHC. Les anticorps anti-VHC peuvent être indétectables à différents stades de l'infection et dans certains états cliniques[27].
- Des anticorps hétérophiles présents dans des échantillons de sérum ou de plasma peuvent être à l'origine d'interférences lors de tests immunologiques[28]. Ces anticorps peuvent être présents dans les prélèvements sanguins de sujets régulièrement en contact avec des animaux ou ayant été traités par des produits sériques d'origine animale. Les résultats incohérents avec les observations cliniques révèlent la nécessité d'analyses supplémentaires.
- Les échantillons contenant de la trioléine (<33,9 mmol/L), de l'hémoglobine (<5 g/L) ou de la bilirubine (<0,342 mmol/L) n'interfèrent pas avec l'interprétation clinique de ces résultats. Ne pas utiliser d'échantillons troubles.

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Interprétation des résultats

Un échantillon déclaré "limite" ou positif doit être retesté en double afin de confirmer les résultats. Avant le nouveau test, ces échantillons doivent être centrifugés afin de garantir une séparation adéquate des cellules, des débris cellulaires et de la fibrine. Si les résultats des tests répétés sont <0,90 pour les deux doublons, les échantillons doivent être considérés comme négatifs. S'ils sont ≥0,90 pour l'un des échantillons, de nouveaux tests doivent être réalisés pour confirmation. Un échantillon positif confirmé par d'autres tests doit être considéré comme positif en anticorps anti-VHC. En cas de résultats "limites" lors des tests supplémentaires, une analyse des échantillons de suivi est recommandée.

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Performances du test

Etalonnage et traçabilité de l'étalonnage
L'étalonnage du test de l'anticorps Anti-VHC est défini en regard d'un étalon de référence interne dont la valeur a été établie de manière à optimiser les performances de sensibilité clinique et de spécificité.

Précision
La précision a été évaluée à l'aide d'une méthode basée sur le protocole EP5-T2[®] du «National Committee for Clinical Laboratory Standards». Deux doublons de 4 échantillons d'un panel ont été testés une fois par jour pendant au moins 20 jours. L'expérience a été effectuée en utilisant 2 lots de réactifs sur des Systèmes différents. Les résultats obtenus sont représentatifs des performances du produit.

Tableau 1 : Précision

Résultat représentatif	Intra-test écart type*	CV(%)*	Intra-étalonnage écart type*	CV(%)*	Intra-laboratoire écart type*	CV(%)*
0,14	0,00336	2,4	0,00835	4,9	0,00945	6,9
2,04	0,044	2,1	0,132	6,4	0,134	6,6
5,74	0,132	2,4	0,339	5,8	0,354	6,2
14,9	0,187	1,2	0,454	3,1	0,576	3,9

* Valeur moyenne quadratique (RMS)

Intra-test : Précision moyenne inter-doublons sur toutes les réactions du jour.
Intra-étalonnage : Précision totale, avec composants pondérés de la variation intra-test et inter-jours.
Intra-laboratoire : Une mesure de l'influence du ré-étalonnage sur la précision totale, calculée sur un lot de réactifs, en utilisant les données de 4 étalonnages.

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Sensibilité
435 échantillons de patients préalablement déclarés positifs par un test VHC immunoblot ont été testés à l'aide du test *Vitros* anti-VHC. La sensibilité du test *Vitros* anti-VHC a été de 100% (435/435) pour cette population d'échantillons.

FR

De plus, 29 panels d'échantillons de séroconversion actuellement disponibles dans le commerce ont été testés. La sensibilité en séroconversion du test *Vitros* Anti-VHC s'est avérée équivalente, voire supérieure, pour les 29 panels comparée à des résultats de tests publiés obtenus par une autre technique disponible dans le commerce.

Spécificité
Des échantillons prélevés sur 5 374 donneurs présumés sains et 393 échantillons cliniques ont été testés à l'aide de la trousse *Vitros* Anti-VHC et d'une méthode disponible dans le commerce.

Echantillons	Nbre d'échant testés	Positif initial	Positif répétable	Positif confirmé
Donneur	5 374	14	13	0
Clinique	393	1	1	0

La spécificité du test *Vitros* anti-VHC pour la population de donneurs a été évaluée à 99,76% (5 361/5 374) en se basant sur les réactivités répétées. La spécificité du test *Vitros* anti-VHC pour la population de donneurs a été évaluée à 99,75% (392/393) en se basant sur les réactivités répétées.

De plus, 161 échantillons provenant de sous-groupes susceptibles de présenter une réactivité croisée ont également été testés à l'aide du test *Vitros* Anti-VHC : échantillons positifs pour les anticorps du CMV, du virus Epstein-Barr et du VIH, ainsi que pour le facteur rhumatoïde, échantillons provenant de patients présentant des hépatopathies non virales ou d'autres hépatopathies virales (VHB, VHA), SLE, échantillons de patients récemment vaccinés (contre la grippe par ex.), échantillons réactifs à la levure. Aucun échantillon de ces sous-groupes n'a présenté de faux positifs avec le test *Vitros* anti-VHC.

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Groupe de travail de l'Agence du Médicament : résultat des études

La trousse *Vitros* Anti-VHC a été évaluée par le groupe de travail de l'Agence du Médicament. 450 échantillons provenant de 246 patients infectés par différents génotypes du virus de l'hépatite C et 50 échantillons du panel VHC SFTS, dont 27 positifs pour l'ARN, ont été testés. Le test *Vitros* Anti-VHC a présenté une bonne sensibilité pour la détection précoce des séroconversions et le dépistage des autres catégories d'échantillons positifs, incluant les échantillons présentant une réactivité isolée et provenant de porteurs du virus.

2018 échantillons de donneurs de sang ont été testés à l'aide de la trousse *Vitros* Anti-VHC. Sur les 8 échantillons positifs répétables, un seul a été confirmé positif, 6 ont été trouvés négatifs avec d'autres tests. Le résultat de l'échantillon restant n'a pu être interprété (NS3 isolée, PCR négative). La spécificité de la trousse *Vitros* Anti-VHC est de l'ordre de 99,70%.

Licence

Les antigènes recombinants du VHC utilisés dans le test anti-VHC *Vitros* sont préparés sous brevet américain par Chiron Corporation dans le cadre d'un contrat de fabrication commune.

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Vitros Immunodiagnostic Products
Anti-HCV Reagent Pack

DE

Zweckbestimmung

Zur qualitativen In-vitro-Diagnostik von Antikörpern gegen Hepatitis-C-Virus (Anti-HCV) in Humanserum und -plasma (EDTA, Heparin oder Zitrat).

Zusammenfassende Erläuterung

Das Hepatitis-C-Virus (HCV) ist die Ursache für die meisten, wenn nicht alle, durch Blut übertragenen Non-A-Non-B-Hepatitiden (NANBH). Auf der ganzen Welt durchgeführte Studien zeigen, dass HCV durch kontaminiertes Blut und Blutprodukte, durch Bluttransfusionen oder durch enge Körperkontakte übertragen wird. Das Vorhandensein von Anti-HCV zeigt an, dass der Proband mit HCV infiziert worden ist und eine HCV-Infektion übertragen könnte[1].

Im *Vitros* Anti-HCV-Test werden drei rekombinante Hepatitis-C-Virus-codierte Antigene verwendet. Die drei rekombinanten Antigene sind c22-3, c200 und NS-5. Das rekombinante Protein c22-3 wird durch die mutmaßliche Core-Region des HCV-Genoms codiert. Das rekombinante HCV-Protein c200 wird durch die mutmaßlichen NS3- und NS4-Regionen des HCV-Genoms codiert. Das c200-Protein enthält die c33c-Proteinsequenz, die genetisch mit der c100-3-Proteinsequenz verbunden ist. Studien haben gezeigt, dass Antikörper, die sich nach einer Infektion mit HCV entwickeln, oft mit c22-3 und/oder c33c reagieren[2]. Das rekombinante HCV-Protein NS5 wird von der mutmaßlichen NS5-Region des HCV-Genoms codiert. Ein signifikanter Anteil der mit HCV infizierten Personen entwickelt Antikörper gegen NS5[3].

Der Wirtsorganismus für alle drei rekombinanten HCV-Antigene ist *S. cerevisiae* (Hefe).

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Prinzip des Verfahrens

Der *Vitros* Anti-HCV-Test wird mit dem *Vitros* Anti-HCV-Reagent Pack und *Vitros* Immunodiagnostic Product Anti-HCV-Calibrator auf dem *Vitros* Immunodiagnostic System durchgeführt.

Es wird eine immunometrische Technik verwendet, bei der eine Zwei-Stufen-Reaktion beteiligt ist. In der ersten Stufe bindet der in der Probe vorhandene HCV-Antikörper an die rekombinanten HCV-Antigene, mit denen die Wells beschichtet sind. Ungebundenes Probenmaterial wird durch Waschen entfernt. In der zweiten Stufe bindet mit Meerrettichperoxidase (HRP) markiertes Antikörperkonjugat (monoklonales Maus anti-human-IgG) an sämtliches humanes IgG, das in der ersten Stufe von den Wells eingefangen wurde. Ungebundenes Konjugat wird durch Waschen entfernt.

Das gebundene HRP-Konjugat wird mittels einer Lumineszenzreaktion bestimmt[4]. Ein Reagenz, das luminogene Substrate (ein Luminolderivat und ein persaures Salz) und ein Elektronentransportmittel enthält, wird in die Wells gegeben. Das HRP im gebundenen Konjugat katalysiert die Oxidation des Luminolderivats, wobei Licht entsteht. Das Elektronentransportmittel (ein substituiertes Acetanilid) erhöht die Stärke des produzierten Lichtsignals und verlängert dessen Emissionsdauer. Die Lichtsignale werden vom *Vitros* System gemessen. Die Menge des gebundenen HRP-Konjugats ist direkt proportional zur Konzentration des in der Probe vorhandenen Anti-HCV.

Gefahrenhinweise und Vorsichtsmaßnahmen

Nur zur In-vitro-Diagnostik

Warnhinweis - Potenziell infektiöses Material

- Diese Materialien sind als potenziell infektiös zu behandeln.
- Proben oder Testkomponenten sollen daher entsprechend den jeweiligen Richtlinien der nationalen Gesundheitsbehörden zum Umgang mit infektiösem Material behandelt, gelagert und entsorgt werden (z.B. NCCLS-Richtlinie M29[5]).

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Der *Vitros* Anti-HCV Calibrator enthält:
HCV-Antikörper-positives Plasma von Spendern, deren Blut individuell mit geeigneten Methoden (EIA, Enzymimmunoassay) Hepatitis-B-Surfaceantigen (HBsAg) und auf Antikörper gegen humane Immunschwächeviren (HIV 1+2) getestet und als nicht reaktiv befunden wurde. Das HCV-Antikörper-positive Plasma wurde behandelt, um den Titer des potenziell infektiösen Virus zu reduzieren. Da jedoch kein Testverfahren das Risiko einer potentiellen Infektion ausschließen kann, ist das Material als potentiell infektiös zu behandeln.

DE

HCV-Antikörper-negatives Plasma stammt von Spendern, deren Blut individuell mit geeigneten Methoden (EIA, Enzymimmunoassay) auf Hepatitis-B-Surfaceantigen (HBsAg) und auf Antikörper gegen HCV und HIV 1+2 getestet und als nicht reaktiv befunden wurde.

Humanserum- oder -plasmaproben sollten immer mit Vorsicht behandelt werden. Alle Proben sollten grundsätzlich als potenziell infektiös angesehen werden. Durch keine der derzeit bekannten Testmethoden kann das Vorhandensein von Hepatitis-B Viren, HCV, HIV 1+2 oder anderen infektiösen Erregern mit absoluter Sicherheit ausgeschlossen werden.

Gefahrstoffhinweis - Enthält ProClin 300 und 2-Chloracetamid

Das Konjugatreagenz enthält ProClin 300 und das Testreagenz enthält 2-Chloracetamid. R43: Sensibilisierung durch Hautkontakt möglich. R52/53: Schädlich für Wasserorganismen, kann in Gewässern längerfristig schädliche Wirkungen haben. S24: Berührung mit der Haut vermeiden. S37: Geeignete Schutzhandschuhe tragen.

Packungsinhalt

1 Reagenzpack mit:
- 100 beschichtete Wells (rekombinante Hepatitis-C-Virus-Antigene aus Hefe; beschichtet mit 0,41 µg/Well).
- 18,2 mL Testreagenz (Puffer mit antimikrobiellem Wirkstoff).
- 20,6 mL Konjugatreagenz - (HRP an monoklonalem Anti-human-IgG aus Mäusen, 1,04 ng/Well) in gepuffertem fötalem Kälberserum mit antimikrobiellem Wirkstoff.

Hinweis: Enthält Rinderserumalbumin und fötales Kälberserum.

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Besondere Materialien (nicht im Lieferumfang enthalten)

Vitros Immunodiagnostic System und folgende *Vitros* Immundiagnostikprodukte: Anti-HCV Calibrator (einschließlich Lot-Kalibrationskarte und Testprotokoll-Magnetkarte), Signalreagenz, Universalwaschreagenz, Reagenzpack-Lagerungsbox (optional) mit Trockenmittel.

Vorbereitung und Lagerung der Reagenzien

Das Reagenzpack wird gebrauchsfertig geliefert. Ungeöffnet bei 2-8 °C lagern, nicht einfrieren. Geöffnete Reagenzpacks innerhalb von 8 Wochen nach dem ersten Laden in ein System aufbrauchen; nach dem Verfallsdatum nicht mehr verwenden. Geöffnete Reagenzpacks im System oder bei 2-8 °C in einer fest verschlossenen Reagenzpack - Lagerungsbox mit Trockenmittel lagern.

Der Anti-HCV Calibrator wird gebrauchsfertig geliefert. Ungeöffnet bei 2-8 °C lagern. Nach dem Verfallsdatum nicht mehr verwenden. Nach dem Öffnen bis zu 13 Wochen bei 2-8 °C oder 13 Wochen bei -20 °C lagern (mit nicht mehr als 1 Einfrier-/ Auftauzyklus).

Vorbereitung des Patienten

Es ist keine besondere Vorbereitung des Patienten erforderlich.

Vorbereitung und Lagerung der Proben

Es können Serum- oder Plasmaproben (EDTA, Heparin oder Zitrat) verwendet werden. Die Ergebnisse von Plasmaproben mit Zitrat werden aufgrund der Verdünnung mit dem flüssigen Antikoagulans proportional niedriger ausfallen. Entnehmen Sie Blutproben mit den üblichen Methoden. Die Proben müssen sorgfältig von allem zellulären Material getrennt werden. Andernfalls können falsch erhöhte Ergebnisse auftreten. Serum- und Plasmaproben können bis zu 7 Tage bei 2-8 °C oder 4 Wochen bei -20°C gelagert werden. Wiederholtes Einfrieren und Auftauen ist zu vermeiden.

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Von Probengewinnungshilfen wurde gelegentlich berichtet, dass sie der Integrität bestimmter Analyte schaden und einige methodische Verfahren stören können[8]. Bedingt durch die Vielzahl unterschiedlicher Probengewinnungshilfen kann Ortho-Clinical Diagnostics nicht für jeden Analyt und jede Gewinnungshilfe eine definitive Aussage über mögliche Störeinflüsse machen. Es wird daher empfohlen, dass das Anwenderlabor sich jeweils selbst davon überzeugt, inwieweit *Vitros* Tests und Gewinnungshilfen unter Berücksichtigung der vom Hersteller empfohlenen Gebrauchsanweisung kompatibel sind.

DE

Qualitätskontrolle und Hinweise zur Verfahrensweise

- Reagenzpack vorsichtig handhaben. Folgendes vermeiden: Kondensatbildung auf dem Pack, Schaumbildung, Schütteln des Packs.
- Die Kalibrierung ist losspezifisch; zusammengehörige Reagenzpacks und Kalibratoren sind mit dem selben Loscode gekennzeichnet. Reagenzpacks des selben Loses können mit der selben Kalibrierung verwendet werden, diese Kalibrierung muss mit einem Kalibrator des selben Loscodes erfolgen.
- Proben, Kalibratoren und Kontrollen vor der Verwendung gründlich durch Umdrehen mischen und auf 15-30 °C bringen.
- Proben, Kalibratoren und Kontrollen in verschlossenen Behältern handhaben, um Kontamination und Verdunstung zu vermeiden. Um eine Verdunstung zu vermeiden, sind die Proben, Kalibratoren und Kontrollen nur für die erforderliche Zeit im *Vitros* System zu belassen. Weitere Informationen finden Sie im Bedienerhandbuch des *Vitros* Systems. Nach dem Gebrauch so bald wie möglich wieder auf 2-8 °C bringen; oder laden Sie nur soviel, wie für eine einzige Bestimmung erforderlich ist. Der Kalibrator kann in alternative Gefäße übertragen werden, die mit den mitgelieferten Barcodeetiketten versehen werden können.
- Der Anti-HCV Kalibrator wird automatisch doppelt analysiert.
- Überprüfen Sie regelmäßig Ihre Vorräte, um das Reagenz-Management zu unterstützen und um sicherzustellen, dass genügend Signalreagenz, Universalwaschreagenz und kalibrierte Reagenzlose für die vorgesehene Arbeit verfügbar sind. Bei der Durchführung von Testprofilen mit einer Probe muss sichergestellt werden, dass das Probenvolumen für die angeforderten Tests ausreicht.

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- Gute Laborpraxis erfordert den Einsatz von Kontrollen, um die Leistungsfähigkeit des Tests zu überprüfen. Es gibt 2 *Vitros* Anti-HCV Kontrollen (Anti-HCV negativ und Anti-HCV positiv). Es wird empfohlen, die Kontrollen nach einer Kalibrierung und dann mindestens alle 24 Stunden sowie nach Durchführung von bestimmten Serviceverfahren zu testen (siehe Bedienerhandbuch des *Vitros* Systems). Folgen Sie den Qualitätskontroll-Richtlinien Ihres Labors, wenn diese ein häufigeres Testen von Kontrollen erfordern. Weitere Informationen finden Sie im Bedienerhandbuch des *Vitros* Systems.
- Der vorgegebene Testname, der auf den Patientenberichten erscheint, ist Anti-HCV. Die vorgegebene Kurzbezeichnung, die auf den Menüs zur Testauswahl und auf den Laborberichten erscheint, ist aHCV. Diese Vorgaben können bei Bedarf mit dem Bildschirm Optionen & Konfiguration - Analyte konfigurieren konfiguriert werden.

Verfahrensweise

Der Test *Vitros* Anti-HCV erfordert 20 µL Proben-, Kalibrator- oder Kontrollmaterial für eine einzelne Messung. Dabei ist das minimale Füllvolumen des gewählten Probengefäßes noch nicht berücksichtigt.

Der Test *Vitros* Anti-HCV muss immer dann kalibriert werden, wenn ein neues Reagenz-Los verwendet wird, danach alle 28 Tage. Eine Kalibrierung des *Vitros* Anti-HCV kann ebenfalls erforderlich werden, nachdem bestimmte Servicemaßnahmen durchgeführt wurden oder wenn die Ergebnisse der Qualitätskontrolle konsistent außerhalb des zulässigen Bereichs liegen.

Ausführliche Anweisungen zur Bedienung des Systems finden Sie im Bedienerhandbuch zum *Vitros* Immundiagnostiksystem, Kapitel 4-7. "Zusammenfassung":

1. Schieben Sie die Testprotokoll-Magnetkarte durch das Lesegerät, um ein neues Testprotokoll ins System zu laden. Das Icon des Tests wird dann unter "Probenprogrammierung" angezeigt. Schieben Sie für jedes neue Reagenz der Los-Kalibrierungskarte durch das Lesegerät, um die Daten über die losspezifische Kalibrierung und das Verfallsdatum einzulesen.

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2. Öffnen Sie die Verpackungsfolie und entnehmen Sie das Reagenzpack. Laden Sie das Pack in die Reagenzien-Ladestation oder verwenden Sie das Icon Entladen/Laden des Menüs "Reagenzmanagement - Ansicht nach Reagenz". **Hinweis:** Verwenden Sie keine beschädigten oder unvollständig verschlossenen Produkte.
3. Laden Sie Proben in Universalprobentrays; verwenden Sie Adapter, falls erforderlich (Proben können bei Bedarf mit Barcodeetiketten versehen werden). Setzen Sie eine Einweg-Pipettierspitze neben jede Probe und laden Sie die Gefäße ins System. Legen Sie Probenprogramme mit dem Bildschirm "Probenprogrammierung" fest. Starten Sie die Testung, alle Stufen der Probenbearbeitung werden automatisch ausgeführt.
4. Kalibratoren werden genauso getestet wie Proben (genügend Material für die automatische Doppelbestimmung laden). Bei Verwendung von Barcodeetiketten braucht die Kalibrierung nicht programmiert zu werden, die Kalibrierung wird automatisch eingeleitet.

DE

Leistungsdaten

Die Ergebnisse werden als normalisierte Messwerte mit Bezug auf einen Cut-Off-Wert berechnet. Während des Kalibrierungsvorgangs wird ein losspezifischer Parameter, der auf der Los-Kalibrierungskarte codiert ist, zur Bestimmung eines gültigen, im System gespeicherten Cut-Off-Wertes verwendet. Weitere Hinweise zur Kalibration sind auf der mit dem Anti-HCV Kalibrator gelieferten Packungsbeilage zu finden. Die Ergebnisse werden automatisch vom *Vitros* System ermittelt.

$$\text{Ergebnis} = \frac{\text{Messwert der getesteten Probe}}{\text{Cut-Off-Wert}}$$

Ein Ergebnis von ≥1,00 zeigt eine reaktive Probe und die Möglichkeit der Anwesenheit von Anti-HCV an.
Ein Ergebnis von <0,90 zeigt eine nicht reaktive Probe an, die für Anti-HCV negativ ist.
Ein Ergebnis von ≥0,90 und <1,00 zeigt eine grenzwertige Probe an.

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Qualitätskontrolle

- Die Kalibrierungsergebnisse werden anhand einer Reihe von Qualitätsparametern überprüft; diese sind einzeln unter der Funktion "Kal.-Parameter einsehen" aufgeführt, die über den Bildschirm "Optionen & Konfiguration - Benutzerkalibrationen einsehen" zugänglich ist. Wenn irgendeiner der festgelegten Qualitätsparameter-Bereiche nicht eingehalten wird, wird dies in dem Kalibrierungsbericht mit einem Code angegeben. Die nach einer misslungenen Kalibrierung durchzuführenden Maßnahmen können unter Kapitel 8, "Ergebnisse einsehen" des Bedienerhandbuchs nachgeschlagen werden.
- Die Werte von Patientenproben werden mit 'Negativ', 'Grenzwertig' oder 'Reaktiv' markiert. Die Werte von Kontrollen werden gekennzeichnet, wenn sie ≥2 SD vom festgelegten Zielwert abweichen.
- Die Qualität der Kalibrierung kann nicht vollständig durch einen einzigen Parameter beschrieben werden. Der Kalibrierungsbericht sollte in Zusammenhang mit Kontrollwerten verwendet werden, um die Gültigkeit der Kalibrierung festzustellen.
- Wenn Kontrollergebnisse außerhalb des zulässigen Bereichs liegen, sind die Gründe dafür festzustellen, bevor eine Entscheidung darüber getroffen wird, ob die Patientenergebnisse mitgeteilt werden sollen.

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Einschränkungen und Störfaktoren

- Die mit diesem oder einem anderen In-vitro-Diagnostikum erhaltenen Ergebnisse sollten stets im Zusammenhang mit dem allgemeinen klinischen Bild des Patienten und anderen diagnostischen Ergebnissen verwendet und interpretiert werden. Ein negatives Testergebnis schließt die Möglichkeit einer Infektion durch HCV nicht mit Sicherheit aus. Bei manchen Stadien der Infektion oder evtl. physiologischen Zuständen können die Anti-HCV-Titer unterhalb der Nachweisgrenze liegen[7].
- Heterophile Antikörper in Serum- oder Plasmaproben können bei Immunoassays[8] Kreuzreaktionen verursachen. Solche Antikörper können in Blutproben von Personen auftreten, die regelmäßig mit Tieren in Kontakt sind oder mit tierischen Serumprodukten behandelt wurden. Ergebnisse, die zu klinischen Beobachtungen im Widerspruch stehen, erfordern zusätzliche Tests.
- Triolein (<33,9 mmol/L), Hämoglobin (<5 g/L) oder Bilirubin (<0,342 mmol/L) in den Proben stört die klinische Interpretation der Ergebnisse nicht. Verwenden Sie keine trüben Proben.

DE

Interpretation der Ergebnisse

Eine grenzwertige oder reaktive Probe muss als Duplikat erneut getestet werden, um ihren Status zu bestätigen. Vor dem erneuten Testen sollte die Probe zentrifugiert werden, um sicherzustellen, dass sie frei von Zellen, Zellbestandteilen und Fibrin ist. Wenn die Ergebnisse bei wiederholtem Testen bei beiden Wiederholungen <0,90 betragen, sollte die Probe als negativ betrachtet werden. Wenn das Testergebnis einer der Wiederholungen ≥0,90 ist, sollte die Probe mit weiteren Tests getestet werden, um das Ergebnis zu bestätigen. Eine wiederholt reaktive Probe, die durch zusätzliche Tests bestätigt wurde, sollte als Anti-HCV-positiv angesehen werden. Bei wiederholt im Grenzbereich liegenden Ergebnissen wird die Untersuchung weiterer Proben empfohlen.

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Technische Angaben

Kalibrierung und Rückverfolgbarkeit der Kalibrierung
Die Kalibrierung des *Vitros* Anti-HCV-Tests ist anhand eines internen Referenzkalibrators nachverfolgbar, der mit echten Messwerten korreliert wurde, um die klinische Sensitivität und Spezifität zu optimieren.

Präzision
Zur Beurteilung der Präzision wurde eine auf dem Protokoll EP5-T2[®] des National Committee for Clinical Laboratory Standards basierende Methode verwendet: Zwei Wiederholungen von jeweils 4 gefriergetrockneten Kontrollseren wurden an mindestens 20 verschiedenen Tagen einmal pro Tag getestet. Das Experiment wurde mit 2 Reagenzlosen auf verschiedenen Systemen durchgeführt. Die hier dargestellten Daten sind für die Leistung des Produkts charakteristisch.

Tabelle 1: Präzision

Repräsentative Ergebnis	Intralauf SD*	CV(%)*	Intrakalibrierung SD*	CV(%)*	Intralabor SD*	CV(%)*
0,14	0,00336	2,4	0,00835	4,9	0,00945	6,9
2,04	0,044	2,1	0,132	6,4	0,134	6,6
5,74	0,132	2,4	0,339	5,8	0,354	6,2
14,9	0,187	1,2	0,454	3,1	0,576	3,9

* Wurzel des Mittelwerts der Quadrate (RMS)

Intralauf: Präzision von Wiederholungsbestimmungen, die über alle Läufe gemittelt wurde.
Intrakalibrierung: Gesamtpräzision, mit gewichteten Anteilen der Intralauf- und Tag-zu-Tag-Variation.
Intralabor: Ein Messwert für die Auswirkung erneuter Kalibrierung auf die Gesamtpräzision, berechnet pro Reagenzlos, mit Daten von 4 Kalibrierungen.

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Sensitivität
435 Patientenproben, die vorher mit einem HCV-Immunoblot-Test als positiv befunden wurden, wurden mit dem *Vitros* Anti-HCV-Test getestet. Die Sensitivität für diese Probenpopulation mit dem *Vitros* Anti-HCV-Test wurde berechnet als 100% (435/435).

Zusätzlich wurden auch noch 29 kommerzielle Serokonversionspanels getestet. Der *Vitros* Anti-HCV Test zeigte bei 29/29 Panels gleiche oder bessere Serokonversions-Sensitivität im Vergleich zu publizierten Daten eines kommerziell erhältlichen HCV-Tests.

DE

Spezifität
Es wurden Proben von 5 374 als gesund angenommenen Blutspendern und 393 klinische Proben mit dem *Vitros* Anti-HCV Test und einem weiteren, kommerziellen Test getestet.

Proben	Anzahl der getesteten Proben	Anfänglich reaktiv	Wiederholt reaktiv	Bestätigt positiv
Spender	5 374	14	13	0
Klinik	393	1	1	0

Die Spezifität des *Vitros* Anti-HCV-Tests für die Spenderpopulation wurde berechnet als 99,76% (5 361/5 374), beruhend auf wiederholt reaktiven Proben. Die Spezifität der *Vitros* Anti-HCV-Tests bei der klinischen Population wurde berechnet als 99,75% (392/393), beruhend auf wiederholt reaktiven Proben.

Zusätzlich wurden 161 Proben von folgenden potenziell kreuzreaktiven Personengruppen mit dem *Vitros* Anti-HCV Test getestet: CMV-positiv, EBV-positiv, HIV positiv, nicht-virale Leberkrankheiten, sonstige virusbedingte Leberkrankheiten (z. B. HBV, HAV), SLE, Rheumafaktor-positiv, kürzliche Impfung (z. B. Grippe), Hefe-reaktive Proben. Von diesen Kategorien rief keine falsch reaktive Ergebnisse mit dem *Vitros* Anti-HCV-Test hervor.

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ADM-Arbeitsgruppe: Zusammenfassung der Bewertung

Der *Vitros* Anti-HCV-Assay wurde von der ADM-Arbeitsgruppe bewertet. 450 Proben von 246 Patienten, die mit verschiedenen Genotypen des Hepatitis-C-Virus infizierten waren, und 50 Proben des HCV-SFTS-Panels - einschließlich 27 RNS-reaktiven Proben - wurden getestet. Der *Vitros* Anti-HCV-Assay zeigte eine gute Sensitivität für den Nachweis der frühen Serokonversion und beim Screening reaktiver Proben anderer Kategorien, einschließlich derer mit isolierter Reaktivität und solcher von chronisch Erkrankten.

Es wurde eine Probengesamtheit von 2018 Blutspenden mit dem *Vitros* Anti-HCV-Assay getestet. Bei acht Proben ergab sich ein wiederholt reaktives Ergebnis, wovon 1 als reaktiv bestätigt wurde und sechs in anderen Assays ein negatives Ergebnis zeigten. Das Ergebnis der restlichen Probe konnte nicht eindeutig festgelegt werden (nicht eindeutig NS3, PCR-negativ). Die Spezifität des *Vitros* Anti-HCV-Assay betrug etwa 99,70%.

Lizenzhinweis

Die im *Vitros* Anti-HCV-Test verwendeten rekombinanten HCV Antigene werden unter U.S.-Lizenz von Chiron Corporation und einer gemeinsamen Herstellungsvereinbarung produziert.

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Vitros Immunodiagnostic Products
Confezione dei reagenti Anti-HCV

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Impiego

Per la misura qualitativa *in vitro* di anticorpi del virus dell'epatite C (anti-HCV) nel siero e nel plasma umani (EDTA, eparina o citrato).

Compendio e spiegazione del dosaggio

Il virus dell'epatite C (HCV) è ormai noto come l'agente che provoca la maggior parte (se non tutte) delle epatiti non-A, non-B (NANBH) a trasmissione ematica. Studi a livello mondiale indicano che l'HCV viene trasmessa attraverso sangue ed emoderivati contaminati, trasfusioni di sangue o contatti personali. La presenza di anti-HCV indica che l'individuo può essere stato infettato con l'HCV e può trasmettere infezione da HCV[1].

Nel dosaggio Anti-HCV *Vitros* vengono utilizzati tre antigeni ricombinanti corrispondenti alla sequenza del virus dell'epatite C: c22-3, c200 e NS-5. La proteina ricombinante c22-3 è codificata dalla regione centrale (core) putativa del genoma HCV. La proteina ricombinante dell'HCV c200 è codificata dalle regioni putative NS3 e NS4 del genoma HCV. La proteina c200 contiene la sequenza della proteina c33c che è geneticamente collegata alla sequenza della proteina c100-3. Dagli studi condotti risulta che gli anticorpi che si sviluppano dopo infezione da HCV sono spesso reattivi con c22-3 e/o c33c[2]. La proteina ricombinante dell'HCV NS5 è codificata dalla regione putativa NS5 del genoma HCV. Una notevole percentuale di persone infette da HCV sviluppano anticorpi all'NS5[3].

L'organismo ospite per tutti e tre gli antigeni ricombinanti dell'HCV è *S. cerevisiae* (lievito).

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Principi della procedura

Il dosaggio Anti-HCV *Vitros* viene eseguito con la confezione di reagente Anti-HCV *Vitros* e il calibratore Anti-HCV per prodotti immunodiagnostici *Vitros* sul sistema immunodiagnostico *Vitros*.

Si utilizza una tecnica immunometrica che implica una reazione in due fasi. Nella prima fase l'anticorpo HCV presente nel campione si lega agli antigeni ricombinanti dell'HCV che rivestono i pozzetti. Il campione non legato viene rimosso mediante lavaggio. Nella seconda fase il coniugato anticorpo marcato con HRP (IgG anti-umane monoclonali di ratto) si lega alle IgG umane catturate sul pozzetto nella prima fase. Il coniugato non legato viene rimosso mediante lavaggio.

Il coniugato HRP legato viene misurato mediante reazione luminescente[4]. Ai pozzetti vengono aggiunti reagente contenente substrati luminogenici (derivato del luminolo e sale peracido) ed agente di trasferimento degli elettroni. L'HRP presente nel coniugato legato catalizza l'ossidazione del derivato del luminolo, producendo luce. L'agente di trasferimento degli elettroni (acetanilide sostituito) aumenta il livello di luce prodotto, prolungandone altresì l'emissione. I segnali luminosi vengono letti dal sistema *Vitros*. Il quantitativo di coniugato HRP legato è direttamente proporzionale al livello di anti-HCV presente.

Avvertenze e precauzioni

Solo per uso diagnostico *in vitro*

Avvertenza - Materiale potenzialmente infettivo

- Trattare come prodotto potenzialmente infettivo.
- Campioni e componenti del dosaggio devono essere manipolati, conservati ed eliminati in forma solida o liquida in conformità alle procedure dettate dalle relative direttive e normative nazionali in materia di sicurezza biologica (per es. Direttiva M29 del NCCLS[5]).

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Il calibratore Anti-HIV 1+2 *Vitros* contiene:
Plasma positivo all'anticorpo HCV ottenuto da donatori sottoposti individualmente ad analisi e risultati negativi all'antigene di superficie dell'epatite B e agli anticorpi del virus dell'immunodeficienza umana (HIV 1+ 2), tramite metodiche approvate (immunosaggi enzimatici). Il plasma positivo all'anticorpo HCV è stato trattato al fine di ridurre il titolo del virus potenzialmente infettivo. Tuttavia, poiché nessun metodo di dosaggio è in grado di escludere il rischio di una potenziale infezione, trattare come agente potenzialmente infettivo.

Plasma negativo all'anticorpo HCV ottenuto da donatori sottoposti individualmente ad analisi e risultati negativi all'antigene di superficie dell'epatite B e agli anticorpi HCV e HIV 1+2, usando metodi approvati (immunodosaggi enzimatici).

Si raccomanda di usare la dovuta cautela nella manipolazione di materiali di origine umana. Tutti i campioni sono da considerare come potenziale fonte di infezione. Nessun metodo sperimentale può garantire con assoluta certezza l'assenza del virus dell'epatite di tipo B, HCV, HIV 1+2 e di altri agenti infettivi.

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Avvertenza - Contiene ProClin 300 e 2-Chloroacetamide
Il reagente coniugato contiene ProClin 300 e reagente di dosaggio contiene 2-Chloroacetamide. R43: Può provocare sensibilizzazione per contatto con la pelle. R52/53: Nocivo per gli organismi acquatici, può provocare a lungo termine effetti negativi per l'ambiente acquatico. S24: Evitare il contatto con la pelle. S37: Usare guanti adatti.

Materiali forniti

L'unità reagenti è costituita da:
- 100 pozzetti rivestiti (antigeni ricombinanti del virus dell'epatite C derivati da lievito; rivestiti a 0,41 µg/pozzetto).
- 18,2 mL di reagente di dosaggio (soluzione tampone con agente antimicrobico).
- 20,6 mL di reagente coniugato - (HRP- IgG anti-umane monoclonali di ratto, 1,04 ng/pozzetto) in siero bovino fetale tamponato con agente anti-microbico.

Nota: Contiene sieroalbumina bovina e siero bovino fetale.

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Materiali richiesti, ma non forniti nel kit

Sistema immunodiagnostico *Vitros* e i seguenti prodotti immunodiagnostici *Vitros*: Anti-HCV Calibratore (comprese la scheda di calibrazione del lotto e la scheda del protocollo), il reagente di segnale, il reagente di lavaggio universale, scatola di conservazione della confezione di reagenti (opzionale) con essiccante.

Preparazione e conservazione dei reagenti

La confezione dei reagenti viene fornita pronta per l'uso. Conservare i reagenti in condizioni integre a 2-8 °C. Non congelare. Una volta aperta, utilizzare la confezione dei reagenti entro 8 settimane dal primo caricamento sul sistema. Non usare oltre la data di scadenza. Conservare le confezioni di reagenti aperte direttamente sul sistema o a 2-8 °C in apposito contenitore ermetico per la conservazione delle confezioni di reagenti sigillati con essiccante a secco.

Il calibratore Anti-HCV viene fornito pronto per l'uso. Conservare in condizioni integre a 2-8 °C. Non usare oltre la data di scadenza. Dopo l'apertura conservare per un massimo di 13 settimane a 2-8 °C o 13 settimane a -20 °C (non è ammesso più di 1 ciclo di congelamento-scongelamento).

Preparazione del paziente

Non è necessaria alcuna preparazione specifica del paziente.

Raccolta, preparazione e conservazione dei campioni

Si possono utilizzare campioni di siero o plasma (EDTA, eparina e citrato). I risultati ottenuti con campioni plasmatici (citrato) saranno proporzionalmente inferiori a causa della diluizione con anticoagulante liquido. Raccogliere i campioni ematici con procedure standard. I campioni devono essere accuratamente separati da tutto il materiale cellulare, altrimenti si rischia di ottenere risultati falsamente elevati. I campioni di siero e plasma possono essere conservati per un massimo di 7 giorni a 2-8 °C o 4 settimane a -20 °C. Evitare congelamenti e scongelamenti ripetuti.

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Si è riscontrato che alcuni dispositivi di prelievo del campione biologico hanno determinato alterazioni all'integrità di determinati analiti provocando interferenze nella determinazione analitica[9]. Considerata la varietà di dispositivi per la raccolta dei campioni disponibili in commercio, la Ortho-Clinical Diagnostics non è in grado di definire con assoluta certezza le prestazioni dei propri prodotti con tutti i diversi dispositivi. Si raccomanda pertanto che i singoli utilizzatori verifichino che il sistema di prelievo da loro adottato venga usato nel rispetto delle istruzioni della Casa produttrice e sia compatibile con il sistema *Vitros*.

Note sul controllo di qualità e sulla procedura

- Maneggiare la confezione dei reagenti con cura evitando la formazione di condensa, evitando la formazione di schiuma nei reagenti ed infine cercando di non agitare la scatola stessa.
- La calibrazione è specifica per i singoli lotti; i calibratori e le confezioni di reagenti vengono associati in base al numero di lotto. Le confezioni di reagenti dello stesso lotto possono utilizzare la stessa calibrazione, che dev'essere effettuata con un calibratore con lo stesso numero di lotto.
- Mescolare perfettamente i campioni, i calibratori e i controlli per inversione e portarli a 15-30 °C prima dell'uso.
- Manipolare i campioni, calibratori e controlli in contenitori chiusi al fine di evitare contaminazione ed evaporazione. Per evitare l'evaporazione, limitare il numero di campioni, calibratori e controlli presenti sul sistema *Vitros*. Per maggiori informazioni vedere la Guida dell'operatore del sistema *Vitros*. Riportare a 2-8 °C quanto prima dopo l'uso oppure caricarne soltanto un quantitativo sufficiente per un singolo impiego. Il calibratore può essere suddiviso/possono suddivisi in aliquote in contenitori alternativi, che devono essere identificati mediante le etichette con codice a barre fornite in dotazione.
- Il calibratore Anti-HCV viene elaborato automaticamente in duplicato.

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- Verificare periodicamente il materiale a disposizione assicurando la corretta gestione dei reagenti e la disponibilità di lotti di reagenti di Segnale, Reagenti di Lavaggio Universale e Reagenti calibrati sufficienti a condurre le analisi programmate. Dovendo eseguire una serie di dosaggi su un singolo campione, è opportuno accertarsi che il volume di campione sia sufficiente ad effettuare i dosaggi richiesti.
- La buona prassi di laboratorio richiede l'esecuzione di controlli per la verifica delle prestazioni del saggio. Sono disponibili 2 controlli Anti-HCV *Vitros* (Anti-HCV negativo e Anti-HCV positivo). Si raccomanda l'esecuzione di controlli all'atto della calibrazione e in seguito almeno ogni 24 ore e dopo l'esecuzione delle procedure di controllo previste (Cfr. la Guida dell'operatore del sistema *Vitros*). O ad intervalli più vicini ove richiesto dalle procedure di controllo qualità in uso presso il laboratorio. Per maggiori informazioni vedere la Guida dell'operatore del sistema *Vitros*.
- Il nome predefinito del dosaggio che appare sul referto del paziente è Anti-HCV. L'abbreviazione predefinita che appare sui menu di selezione dei dosaggi e sui referti di laboratorio è aHCV. Se necessario è possibile riconfigurare questi valori predefiniti dalla videata di configurazione degli analiti nelle Opzioni e Configurazioni.

Procedura

Il dosaggio per Anti-HCV *Vitros* richiede 20 µL di campione, calibratore o controllo per una determinazione singola, indipendentemente dal volume minimo di riempimento del contenitore per campioni utilizzato.

Il dosaggio per Anti-HCV *Vitros* dev'essere calibrato ogni volta si usi un nuovo lotto di reagenti e, successivamente, ad intervalli di 28 giorni. Il dosaggio per Anti-HCV *Vitros* dev'essere calibrato anche dopo l'esecuzione di alcune procedure di controllo oppure se i risultati del controllo qualità differiscono molto dal range accettabile.

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Per informazioni più dettagliate sul funzionamento del sistema si veda la Guida dell'Operatore del Sistema immunodiagnostico *Vitros*, capitoli 4-7. In breve:

1. Effettuare la scansione della scheda del protocollo per caricare un nuovo protocollo di analisi sul sistema. A questo punto viene visualizzato il pulsante relativo alle diverse determinazioni sulla videata di programmazione dei campioni. Effettuare la scansione della scheda di calibrazione dei lotti per ogni nuovo lotto di reagenti per immettere i dati di scadenza e di calibrazione specifici per quel lotto.
2. Aprire la busta e prelevare la confezione dei reagenti. Caricare la confezione sull'unità di autocaricamento o utilizzare il pulsante di scarico/carico nella videata di gestione dei reagenti (visualizzazione per reagente). **Nota:** Non utilizzare prodotti danneggiati o non sigillati perfettamente.
3. Caricare i campioni nei settori portacampioni universali usando, se necessario, appositi adattatori (volendo, si possono identificare i campioni con codici a barre). Collocare un puntale monouso accanto ai singoli campioni e caricare i settori sul sistema. Definire i programmi dei campioni tramite la videata di programmazione dei campioni. Avviare la funzione di campionamento. Tutte le fasi di campionamento vengono effettuate automaticamente.
4. Analizzare il calibratore come se si trattasse di campioni (caricare un quantitativo sufficiente per la misura automatica in duplicato), Se si usano etichette con codici a barre non occorre programmare la calibrazione; la calibrazione verrà effettuata automaticamente.

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Risultati

I risultati sono calcolati come un segnale normalizzato, relativo ad un valore di cut-off. Durante la procedura di calibrazione viene utilizzato un parametro specifico del lotto, codificato sulla scheda di calibrazione dei lotti, ai fini della determinazione di un valore di cut-off valido per il sistema. Per maggiori informazioni sulla calibrazione vedere l'inserto illustrativo contenuto nel calibratore Anti-HCV. I risultati vengono calcolati automaticamente dal Sistema *Vitros*.

$$\text{Risultato} = \frac{\text{Segnale per il campione di prova}}{\text{Valore di cut-off}}$$

Un risultato di ≥1,00 indica un campione reattivo e l'eventuale presenza di Anti-HCV.
Un risultato di <0,90 indica un campione non reattivo, negativo per Anti-HCV.
Un risultato di ≥0,90 e <1,00 indica un campione borderline.

Controllo di qualità

- I risultati delle calibrazione vengono valutati a fronte di due parametri di qualità secondo quanto indicato nella Visualizzazione dei parametri di calibrazione, cui si accede dalla videata Opzioni e configurazione - Revisione/Calibrazioni dell'utente. Il mancato rispetto dei parametri di qualità definiti viene codificato nel rapporto di calibrazione. Per le azioni da mettere in atto in seguito ad una calibrazione non andata a buon fine, vedere la Guida dell'operatore, capitolo 8, Revisione dei risultati.
- I valori del campione dei paziente vengono identificati come 'Negativo', 'Borderline' o 'Reattivo'. I valori di controllo vengono identificati se la DS ≥2 rispetto alla media di baseline definita.
- La qualità della calibrazione non può essere descritta appieno da un singolo parametro. Il rapporto di calibrazione dev'essere utilizzato in abbinamento ai valori di controllo ai fini della determinazione della validità della calibrazione.
- Se i risultati di controllo differiscono di molto dal range accettabile, indagarne la causa prima di decidere se annotare i risultati del paziente.

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Limiti della procedura

- I risultati ottenuti con questo o altri kit diagnostici devono essere utilizzati ed interpretati esclusivamente nel contesto del quadro clinico generale. Un risultato negativo al test non esclude la possibilità di un'avvenuta esposizione o infezione da HCV. Gli anticorpi anti-HCV potrebbero essere non rilevabili in alcune fasi dell'infezione o in alcune condizioni cliniche[7].
- Gli anticorpi eterofili nei campioni di siero o di plasma possono provocare interferenze negli immunodosaggi[8]. Questi anticorpi possono essere presenti nei campioni di sangue provenienti da individui a contatto con animali o che sono stati curati con prodotti derivati da siero animale. I risultati che sono contraddittori con le osservazioni cliniche indicano che sono necessari altri test.
- I campioni contenenti trioleina (<33,9 mmol/L), emoglobina (<5 g/L) o bilirubina (<0,342 mmol/L) non interferiscono con l'interpretazione clinica dei risultati. Non usare campioni torbidi.

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Interpretazione dei risultati

Un campione risultato borderline o reattivo deve essere testato nuovamente in duplicato per verificarne lo stato. Prima di essere rianalizzati, i campioni devono essere centrifugati in modo da risultare esenti da cellule, depositi cellulari o fibrina. Se i risultati del secondo test sono <0,90 per i due duplicati, il campione deve essere considerato negativo. Se entrambi i risultati ottenuti nel secondo test sono ≥0,90, si deve risottoporre il campione a test supplementari di conferma. I campioni risultati ripetutamente reattivi nei test supplementari devono essere considerati positivi all'anti-HCV. Nel caso di risultati ripetutamente borderline, si raccomanda di eseguire analisi di follow-up.

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Prestazioni metodologiche

Calibrazione e modalità per ricostruirla
La calibrazione del dosaggio Anti-HCV *Vitros* è ricavabile da un calibratore di riferimento interno che è stato avvalorato per ottimizzare la sensibilità e la specificità clinica.

Precisione
La precisione è stata valutata in conformità al protocollo EP5-T2[6] National Committee for Laboratory Standards. Due replicati per ciascuno di 4 pannelli sono stati dosati in una singola occasione al giorno per almeno 20 giorni diversi. L'esperimento è stato effettuato usando 2 lotti di reagenti su sistemi diversi. I dati riportati sono rappresentativi delle prestazioni del prodotto.

Tabella 1: Precisione

Risultato Rappresentativo	Intra-dosaggio DS*	Intra-dosaggio CV(%)*	Intracalibrazione DS*	Intracalibrazione CV(%)*	Intralaboratorio DS*	Intralaboratorio CV(%)*
0,14	0,00336	2,4	0,00835	4,9	0,00945	6,9
2,04	0,044	2,1	0,132	6,4	0,134	6,6
5,74	0,132	2,4	0,339	5,8	0,354	6,2
14,9	0,187	1,2	0,454	3,1	0,576	3,9

* Quadratica media

Giornaliera: Precisione fra un duplicato e l'altro, con media ottenuta su tutti i replicati.
Intracalibrazione: Precisione totale, con componenti ponderati di variazione infra-replica e l'altra e da un giorno all'altro.
Intralaboratorio: Misura dell'effetto della ricalibrazione sulla precisione totale, calcolata nell'ambito di un singolo lotto di reagenti, utilizzando dati di 4 calibrazioni.

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Sensibilità
435 campioni di pazienti già risultati positivi mediante saggi HCV "immunoblot" sono stati testati con il dosaggio Anti-HCV *Vitros*. La sensibilità per tale popolazione di campioni nel dosaggio Anti-HCV *Vitros* è stata calcolata pari al 100% (435/435).

Inoltre, sono stati testati 29 pannelli di sieroconversione disponibili in commercio. Il dosaggio Anti-HCV *Vitros* ha indicato una sensibilità di sieroconversione equivalente o superiore per 29/29 pannelli, se confrontata con quella riportata su pubblicazioni relative ad un altro dosaggio in commercio.

Specificità
Campioni di 5 374 donatori di sangue in apparente buona salute e 393 campioni clinici sono stati testati con dosaggio Anti-HCV *Vitros* e altro dosaggio del commercio.

IT

Campioni	Numero di campioni analizzati	Inizialmente reattivi	Ripetutamente reattivi	Confermati positivi
Donatore	5 374	14	13	0
Clinici	393	1	1	0

La specificità del dosaggio Anti-HCV *Vitros* per la popolazione di donatori è stata calcolata nell'ordine del 99,76% (5 361/5 374) basandosi su reattivi ripetuti. La specificità del dosaggio Anti-HCV *Vitros* per la popolazione clinica è stata calcolata nell'ordine del 99,75% (392/393) basandosi su reattivi ripetuti.

Inoltre sono stati testati con il dosaggio Anti-HCV *Vitros* 161 campioni dei seguenti sottogruppi potenzialmente cross-reagenti: CMV positivi, EBV positivi, HIV positivi, pazienti con malattie epatiche non virali, altre malattie epatiche virali (p. es., HBV, HAV), SLE, positivi al fattore reumatoide, vaccinati di recente (p. es., vaccino antinfluenzale), campioni reattivi al lievito. Nessuna di tali categorie ha rivelato falsi reattivi nel dosaggio Anti-HCV *Vitros*.

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Gruppo di lavoro ADM: Compendio di valutazione

Il saggio Anti-HCV *Vitros* è stato valutato dal gruppo di lavoro ADM. Sono stati testati 450 campioni provenienti da 246 pazienti risultati positivi alla presenza di diversi genotipi di virus dell'epatite C nonché 50 campioni da campioni rappresentativi SFTS di HCV, compresi 27 campioni reattivi all'acido ribonucleico (RNA). Il saggio Anti-HCV *Vitros* ha presentato una buona sensibilità per la determinazione della sieroconversione precoce e per lo screening di altre categorie di campioni reattivi, inclusi quelli che indicano una reattività isolata e quelli provenienti da portatori cronici.

Una popolazione di 2018 campioni provenienti da donatori di sangue è stata testata con il saggio Anti-HCV *Vitros*. Otto campioni hanno dato risultati ripetibilmente reattivi, tra cui 1 è stato confermato come reattivo e 6 sono invece risultati negativi con altri saggi. Il risultato del campione rimanente non ha potuto essere interpretato (NS3 non determinato, reazione a catena della polimerasi - PCR - negativa). La specificità del saggio Anti-HCV *Vitros* è stata calcolata approssimativamente del 99,70%.

Gli antigeni ricombinanti dell'HCV utilizzati nel dosaggio Anti-HCV *Vitros* vengono preparati su licenza statunitense dalla Chiron Corporation nell'ambito di un accordo di fabbricazione bilaterale.

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Vitros Immunodiagnostic Products
Embalagem de Reagente Anti-HCV

PT

Indicação de Utilização

Para utilização *in vitro* na detecção qualitativa de anticorpos do vírus da Hepatite C (Anti-HCV) em soro e plasma humanos (EDTA, heparina ou citrato).

Sumário e Explicação

O vírus da Hepatite C (HCV) é conhecido como sendo o agente causador da maior parte, se não mesmo da totalidade, das hepatites não-A, não-B (NANBH) sanguíneas. Estudos efectuados em todo o mundo indicam que o HCV é transmitido através do sangue e produtos de sangue contaminados, transfusões sanguíneas ou outros contactos mais íntimos. A presença de anti-HCV indica que o indivíduo pode ter sido infectado por HCV e ser capaz de transmitir a infecção[1].

São utilizados três antigénios recombinantes do vírus da Hepatite C no teste Anti-HCV *Vitros*. Estes três antigénios recombinantes são c22-3, c200 e NS-5. A proteína recombinante c22-3 é codificada pela região putativa do núcleo do genoma do HCV. A proteína recombinante c200 é codificada pelas regiões mutáveis NS3 e NS4 do genoma do HCV. A proteína c200 contém a sequência da proteína c33c que é geneticamente ligada à sequência da proteína c100-3. Estudos efectuados indicaram que os anticorpos que se desenvolvem após a infecção pelo HCV são muitas vezes reactivos para a c22-3 e/ou c33c[2]. A proteína recombinante NS5 é codificada pela região mutável NS5 do genoma do HCV. Uma proporção significativa de pessoas infectadas por HCV desenvolvem anticorpos contra NS5[3].

O organismo hospedeiro para os três antigénios recombinantes do HCV é o *S. cerevisiae* (levedura).

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Princípio do Procedimento

O teste Anti-HCV *Vitros* é realizado utilizando-se a Embalagem de Reagente Anti-HCV *Vitros* e o Calibrador *Vitros* dos Produtos de Imunodiagnóstico Anti-HCV *Vitros* no Sistema de Imunodiagnóstico *Vitros*.

Utiliza-se uma técnica imunométrica que envolve uma reacção de duas etapas. Na primeira etapa, o anticorpo HCV presente na amostra liga-se ao antigénio recombinante do HCV revestido nas paredes dos poços. A amostra não ligada é removida através de lavagem. Na segunda etapa o anticorpo conjugado (monoclonal de rato IgG anti-humano) marcado com HRP (peroxidase de rábano) liga-se ao IgG humano capturado no poço na primeira etapa. O conjugado não ligado é removido através de lavagem.

A ligação conjugado/HRP é medida por uma reacção luminescente[49]. É adicionado aos poços reaccionais um reagente contendo substrato luminogénico (um derivado de luminol e um sal perácido) e um agente transferidor de electrões. A HRP presente na ligação conjugada catalisa a oxidação do derivado de luminol produzindo luz. O agente transferidor de electrões (uma acetanilida derivada) aumenta a intensidade da luz produzida e prolonga a sua emissão. Os sinais de luz produzidos são lidos pelo Sistema *Vitros*. A quantidade de conjugado HRP ligado é directamente proporcional ao nível de anti-HCV presente.

Avisos e Precauções

Para Diagnóstico *In Vitro*

Aviso - Material Potencialmente Infeccioso

- Tratar como passível de transmitir infecções.
- O manuseamento de amostras e de componentes do teste, a sua utilização, armazenamento e eliminação de sólidos e líquidos deverão ser efectuados de acordo com os procedimentos definidos pelos regulamentos nacionais (por exemplo, NCCLS Directriz M29[50]).

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O Calibrador Anti-HCV *Vitros* contém:
Plasma com anticorpo HCV positivo obtido através de dadores testados individualmente e que se revelaram negativos ao antigénio de superfície da Hepatite B e aos anticorpos contra o vírus de imunodeficiência humana (HIV 1+2), usando métodos aprovados (testes imunológicos com enzimas). O plasma com anticorpo HCV positivo recebeu tratamento para reduzir o título de vírus potencialmente infecciosos. Mas como nenhum método de teste é capaz de garantir totalmente a ausência do risco, manipule como se passível de transmitir infecções.

O plasma negativo para o anticorpo face o HCV foi obtido através de dadores testados individualmente e que se revelaram negativos ao antigénio de superfície da Hepatite B e aos anticorpos contra HCV e HIV 1+2, utilizando métodos aprovados (testes imunológicos com enzimas).

Deverão ser tomadas precauções quando se manuseiam materiais de origem humana. Todas as amostras devem ser consideradas como potencialmente infecciosas. Nenhum método de teste pode garantir a ausência total de vírus da Hepatite B, HCV, HIV 1+2 ou outros agentes infecciosos.

Aviso – Contém ProClin 300 e 2-Cloroacetamida
O Reagente Conjugado contém ProClin 300 e o Reagente de Teste contém 2-Cloroacetamida. R43: Pode causar sensibilização em contacto com a pele. R52/53: Nocivo para os organismos aquáticos, podendo causar efeitos nefastos a longo prazo no ambiente aquático. S24: Evitar o contacto com a pele. S37: Usar luvas adequadas.

Material fornecido

1 embalagem de reagente contendo:
- 100 poços revestidos (antigénios recombinantes do vírus da hepatite C derivado de levedura, revestidos a 0,41 µg/poço).
- 18,2 ml de Reagente de Teste (solução tampão com agente antimicrobiano).
- 20,6 ml de Reagente Conjugado - (HRP- monoclonal de rato IgG anti-humano, 1,04 ng/poço) em soro de feto de bezerro tamponado e com agente antimicrobiano.

Observação: Contém albumina sérica bovina e soro de feto de bezerro.

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Material Necessário não Fornecido

Sistema de Imunodiagnóstico *Vitros* e os seguintes Produtos de Imunodiagnóstico *Vitros*: Calibrador Anti-HCV (incluindo cartão magnético de calibração do lote e cartão magnético de protocolo), Reagente de Sinal, Reagente de Lavagem Universal, Caixa de Armazenamento da Embalagem de Reagente (opcional) com excicante.

Preparação e Armazenamento do Reagente

A embalagem de reagente é fornecida pronto a utilizar. Conservar as embalagens fechadas a uma temperatura entre 2-8 °C; não congelar. Após a abertura utilizar as embalagens de reagentes no prazo máximo de 8 semanas após o primeiro carregamento num Sistema; não utilizar após o fim do prazo de validade indicado na embalagem. Conservar as embalagens de reagentes abertas dentro do Sistema ou a uma temperatura entre 2-8 °C numa caixa de armazenamento selada contendo excicante seco.

O Calibrador Anti-HCV é fornecido pronto a utilizar. Conservar fechado a uma temperatura entre 2-8 °C. Não utilizar após o prazo de validade indicado na embalagem. Depois de aberto, conservar a uma temperatura entre 2-8 °C durante 13 semanas ou a um temperatura de -20 °C durante 13 semanas (não ultrapassar mais que 1 ciclo de congelamento/descongelamento).

Preparação do Doente

Não é necessário nenhuma preparação do doente.

Recolha das Amostras, Preparação e Armazenamento

Podem ser utilizadas amostras de soro ou plasma (EDTA, heparina ou citrato). Os resultados das amostras de plasma de citrato serão proporcionalmente mais baixos devido à diluição com o líquido anticoagulante. Fazer as recolhas de sangue de acordo com os procedimentos normalizados. As amostras devem ser separadas completamente de todo o material celular. O não cumprimento desta advertência pode originar resultados falsamente elevados. As amostras de soro e plasma podem ser armazenadas durante 7 dias a uma temperatura entre 2 e 8 °C ou até 4 semanas a -20°C. Evite ciclos de congelação e descongelação constantes.

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Em certas ocasiões registou-se que os instrumentos de recolha de amostras poderiam prejudicar a integridade de alguns analitos e que poderiam interferir com o método de algumas tecnologias[69]. Devido à variedade de meios de recolha de amostras disponíveis, a Ortho-Clinical Diagnostics não pode emitir uma conclusão definitiva sobre a actuação dos seus produtos com estes instrumentos. Recomenda-se que cada utilizador assegure que o meio escolhido é utilizado de acordo com as instruções do fabricante e que é compatível com o teste *Vitros*.

Controlo de Qualidade e Notas de Procedimento

- Manusear a embalagem de reagente com cuidado, evitando a formação de condensação na embalagem, a formação de espuma no seu interior e a agitação da embalagem.
- A calibração é específica para cada lote; as embalagens de reagentes e os calibradores estão ligados pelo número de lote. As embalagens de reagente do mesmo lote utilizam a mesma calibração, que deve ser efectuada utilizando um calibrador do mesmo número de lote.
- Misture cuidadosamente as amostras, o calibrador e os controlos através de inversão e mantenha a uma temperatura entre 15-30 °C antes de utilizar.
- Manusear as amostras, calibradores e controlos em recipientes tapados para evitar a contaminação e a evaporação. Para evitar a evaporação, limitar o tempo das amostras, calibradores e controlos no Sistema *Vitros*. Consultar o Guia do Operador do Sistema *Vitros* para mais informações. Depois da utilização, coloque rapidamente a uma temperatura entre 2-8 °C ou carregar o suficiente para uma única utilização. O calibrador pode ser dividido em contentores alternativos e estes podem ser etiquetados com os códigos de barra fornecidos.
- O Calibrador Anti-HCV é processado em duplicado automaticamente.
- Verifique regularmente o inventário para uma gestão mais eficiente dos reagentes e para garantir que existe Reagente de Sinal *Vitros* e Reagente de Lavagem Universal *Vitros* suficientes e lotes de reagente calibrados disponíveis para o trabalho planeado. Quando realizar conjuntos de testes numa única amostra, certifique-se que o volume da amostra é suficiente para os testes pedidos.

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- As Boas Práticas de Laboratório exigem que os controlos sejam processados para que se possa verificar o desempenho do teste. Existem 2 Controlos Anti-HCV *Vitros* (Anti-HCV negativo e Anti-HCV positivo). Recomenda-se que os controlos sejam processados sempre que se realiza a calibração de um novo lote e, consequentemente, pelo menos uma vez a cada 24 horas e após a utilização de procedimentos de serviço específicos (Consultar o Guia do Operador do Sistema *Vitros*). Se os métodos de controlo dentro do seu laboratório exigem uma utilização mais frequente dos controlos, siga esses procedimentos. Consultar o Guia do Operador do Sistema *Vitros* para mais informações.
- O nome do teste, por defeito, que irá aparecer nos relatórios dos doentes é Anti-HCV. O nome abreviado, por defeito, que irá aparecer nos menus de selecção do teste e nos relatórios laboratoriais é aHCV. Estas características podem ser reconfiguradas, se necessário, no ecrã Options & Configurations - Configure Analytes.

Procedimento

O teste Anti-HCV *Vitros* requer 20 µl de amostra, calibrador ou controlo para uma única determinação. Este valor não inclui o volume mínimo do recipiente de amostra escolhido.

O teste Anti-HCV *Vitros* deve ser calibrado sempre que é utilizado um novo lote de reagente e, subsequentemente, em intervalos de 28 dias. O teste Anti-HCV *Vitros* também pode necessitar de ser calibrado após a utilização de certos procedimentos de serviço ou se os resultados de controlo de qualidade estiverem constantemente fora do seu intervalo de aceitação.

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Para instruções detalhadas sobre o funcionamento do Sistema, consultar o Guia do Operador do Sistema de Imunodiagnóstico *Vitros*, Capítulos 4-7. Resumindo:

1. Passe o cartão magnético de protocolo para introduzir um novo protocolo de teste no Sistema. O botão relativo ao teste aparecerá no ecrã Sample Programming. Passe o cartão magnético de calibração do lote em cada lote de reagente novo de modo a introduzir a calibração específica do lote e os dados sobre a data de validade.
2. Abra a bolsa de alumínio e retire a embalagem de reagente. Coloque a embalagem na estação de auto-carregamento ou utilize o botão Load/Unload no ecrã Reagent Management - View by Reagent. **Observação:** Não utilizar produtos com o selo danificado ou selados parcialmente.
3. Coloque as amostras em tubos universais ou outros recipientes de amostras utilizando adaptadores sempre que necessário (se desejar, as amostras podem ser etiquetadas com código de barras). Coloque uma ponta descartável adjacente a cada amostra e ponha os suportes dentro do Sistema. Defina os programas de amostras utilizando o ecrã Sample Programming. Inicie a operação de pipetação. Todos os passos de processamento das amostras serão realizados automaticamente.
4. Processe o calibrador da mesma maneira que as amostras (carregando o suficiente para a determinação automática em duplicado). A calibração não necessita de ser programada se se utilizarem etiquetas de código de barras, esta será automaticamente iniciada.

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Resultados

Os resultados são calculados como um sinal normalizado, relativo a um valor de cut-off. Durante o processo de calibração é utilizado o parâmetro específico do lote, codificado no cartão de calibração do lote, para determinar para o Sistema um valor de cut-off armazenado válido. Para mais informações sobre a calibração consultar as instruções de uso fornecidas com o Calibrador Anti-HCV. Os resultados são calculados automaticamente pelo Sistema *Vitros*.

$$\text{Resultado} = \frac{\text{Sinal da amostra}}{\text{Valor de Cut-off}}$$

Um resultado ≥1,00 indica uma amostra reactiva e a possível presença de Anti-HCV.
Um resultado <0,90 indica uma amostra não-reactiva, negativa para o Anti-HCV.
Um resultado de ≥0,90 e <1,00 indica uma amostra de fronteira.

Controlo de Qualidade

- Os resultados de calibração são avaliados comparativamente a dois parâmetros de qualidade detalhados em View Cal Parameters, cujo acesso se faz por meio do ecrã Options Configuration - Review/User Calibration. O não cumprimento de todos os parâmetros de qualidade definidos será registado no relatório de calibração. Para acções a serem tomadas após uma calibração falhada, consulte-se o Guia do Operador, capítulo 8, Revisão dos Resultados.
- Os valores das amostras dos doentes são marcados como "Negativo", "Fronteira" ou "Reactivo". Os valores de controlo são marcados sempre que o desvio seja ≥2 DPs da média definida.
- A qualidade da calibração não pode ser totalmente descrita por um único parâmetro. O relatório de calibração deve ser utilizado juntamente com os valores de controlo para que se possa determinar a validade da calibração.
- Se os resultados controlo estiverem fora do intervalo de aceitação, investigar a causa antes de decidir pela utilização ou não dos resultados do doente.

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Limitações do Procedimento

- Os resultados deste ou de qualquer outro kit de diagnóstico devem ser utilizados e interpretados apenas no contexto global do quadro clínico do doente. Um resultado negativo não exclui a possibilidade de exposição ou infecção pelo HCV. Os anticorpos HCV podem ser indetectáveis em algumas fases da infecção e em determinados estados clínicos[7].
- Os anticorpos heterófilos em amostras de soro ou de plasma podem provocar interferências nos imunotestes[8]. Estes anticorpos podem estar presentes em amostras de sangue de indivíduos que estão regularmente em contacto com animais ou que foram tratados com produtos à base de soro animal. Os resultados que não forem consistentes com as observações clínicas indicam a necessidade da realização de testes adicionais.
- Amostras que contenham triolina (<33,9 mmol/L), hemoglobina (<5 g/L) or bilirrubina (<0,342 mmol/L) não interferem com a interpretação clínica destes resultados. Não utilizar amostras turvas.

Interpretação de Resultados

Uma amostra considerada duvidosa ou reactiva deve ser testada novamente em duplicado para verificar a sua condição. Antes de realizar novamente o teste, a amostra deve ser centrifugada para garantir a ausência de células, de fragmentos celulares ou de fibrina. Se os resultados após a repetição do teste forem <0,90 nos dois testes, então a amostra deve ser considerada negativa. Se ambos os testes tiverem um resultado ≥0,90, a amostra deve ser testada através de ensaios suplementares para confirmar o resultado. Uma amostra repetidamente reactiva confirmada por testes suplementares deve ser considerada positiva para o anti-HCV. No caso de resultados repetidamente fronteira, recomenda-se a realização de análises a amostras posteriores.

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Características de Actuação

Calibração e Rastreabilidade de Calibração
A calibração do teste Anti-HCV *Vitros* pode ser rastreada através de um calibrador de referência interno que tenha sido designado para optimizar a sensibilidade clínica e a eficiência específica.

Precisão
A precisão foi avaliada de acordo com o protocolo EP5-T2[RN] do Comité Nacional para os Padrões Clínicos Laboratoriais. Foram testados uma única vez por dia em pelo menos 20 dias diferentes, dois replicados de cada um dos 4 painéis de amostras. O ensaio foi efectuado com 2 lotes de reagente em Sistemas diferentes. Os dados apresentados são representativos da actuação do produto.

Quadro 1: Precisão

Resultado Representativo	Na mesma corrida		Na mesma calibração		No laboratório	
	SD*	CV(%)*	SD*	CV(%)*	SD*	CV(%)*
0,14	0,00336	2,4	0,00835	4,9	0,00945	6,9
2,04	0,044	2,1	0,132	6,4	0,134	6,6
5,74	0,132	2,4	0,339	5,8	0,354	6,2
14,9	0,187	1,2	0,454	3,1	0,576	3,9

* Média da Raíz Quadrada (MRQ)

Na mesma corrida: Precisão entre duplicados, estabelecida a partir da média de todos os parâmetros
Na mesma calibração: Precisão total, com componentes pesados na mesma corrida, variação entre parâmetros e no mesmo dia.
No laboratório: Uma medida do efeito de recalibração na precisão total, calculada no mesmo lote de reagente, utilizando dados de 4 calibrações.

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Sensibilidade
Foram testadas com o teste Anti-HCV *Vitros* 435 amostras de pacientes previamente determinadas como positivas por um teste HCV Immunoblot. A sensibilidade desta população de amostras ao teste Anti-HCV *Vitros* foi calculada como 100% (435/435).

Adicionalmente, foram testados 29 painéis de seroconversão comercialmente disponíveis. O teste Anti-HCV *Vitros* demonstrou uma sensibilidade de seroconversão equivalente ou superior em 29/29 painéis quando comparado com os resultados publicados de outros testes disponíveis comercialmente.

Especificidade
Foram submetidas ao teste Anti-HCV *Vitros* e a outro teste disponível comercialmente 5 374 amostras de dadores de sangue presumivelmente saudáveis e 393 amostras clínicas.

Amostras	Número de Amostras de Teste	Inicialmente Reactivas	Repetidamente Reactivas	Confirmadas Positivas
Dador	5 374	14	13	0
Clínico	393	1	1	0

A especificidade do teste Anti-HCV *Vitros* para a população de dadores foi calculada como sendo 99,76% (5 361/5 374) baseada em amostras repetidamente reactivas. A especificidade do teste Anti-HCV *Vitros* para a população clínica foi calculada como sendo 99,75% (392/393) baseada nas amostras repetidamente reactivas.

Adicionalmente, 161 amostras dos seguintes sub-grupos potencialmente reactivo-cruzados foram testadas com o teste Anti-HCV *Vitros* : CMV positivo, EBV positivo, HIV positivo, pacientes com doenças hepáticas não-virais, outras doenças hepáticas virais (por exemplo, HBV, HAV), SLE, factor reumatóide positivo, vacinados recentes (por exemplo, contra gripe), amostras reactivas a leveduras. Nenhuma dessas categorias revelou resultados falsamente reactivos no teste Anti-HCV *Vitros*.

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Trabalho do Grupo da ADM: Sumário da Avaliação

O teste *Vitros* Anti-HCV foi testado pelo grupo de avaliação da ADM. Foram testadas 450 amostras de 246 doentes infectados com diferentes genótipos do vírus da Hepatite C e 50 amostras do painel HCV SFTS, incluindo 27 amostras RNA reactivas. O teste Anti-HCV *Vitros* demonstrou boa sensibilidade para a detecção de seroconversão adiantada e para a triagem de outras categorias de amostras reactivas, incluindo as com reactividade isolada e de portadores crónicos.

Uma população de 2018 amostras de dadores de sangue foi testada com o teste Anti-HCV *Vitros*. Oito amostras mostraram resultados repetidamente reactivos, dos quais um foi confirmado como reactivo e seis considerados negativos por outros testes. O resultado da amostra restante não pôde ser interpretado (NS3 indeterminado, PCR negativo). A especificidade do teste Anti-HCV *Vitros* foi de aproximadamente 99,70%.

Declaração de Licença

Os antigénios recombinantes do HCV utilizados no teste Anti-HCV *Vitros* são preparados sob licença dos EUA para a Chiron Corporation sob acordo de fabricopartilhado.

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Vitros Immunodiagnostic Products
Kit de Reactivos anti-HCV

Uso previsto

Para la detección cualitativa *in vitro* de anticuerpos frente al virus de la hepatitis C (anti-HCV) en suero y plasma (EDTA, heparina o citrato) humanos.

Resumen y explicación del test

Actualmente se sabe que el virus de la hepatitis C (HCV) es el agente causante de la mayoría, cuando no de todos, los casos de hepatitis no A, no B, transmitida por la sangre. En estudios realizados en todo el mundo se señala que el HCV se transmite mediante la sangre y productos hemoderivados contaminados, mediante transfusiones sanguíneas o mediante otros contactos personales íntimos. La presencia de anticuerpos frente al HCV indica que una persona puede haber sido infectada por el HCV y que puede transmitir la infección por el HCV[1].

ES

En el test *Vitros* anti-HCV se han utilizado tres antígenos codificados recombinantes del virus de la hepatitis C. Los tres antígenos recombinantes son c22-3, c200 y NS-5. La proteína recombinante c22-3 es codificada por la región nuclear putativa del genoma del HCV. La proteína recombinante c200 del HCV es codificada por las regiones putativas NS3 y NS4 del genoma del HCV. La proteína c200 contiene la secuencia de la proteína c33c, que por lo general está genéticamente relacionada con la secuencia de la proteína c100-3. Algunos estudios han indicado que los anticuerpos que se desarrollan tras la infección por el HCV a menudo son reactivos con la proteína c22-3 o con la c33c[2]. La proteína recombinante NS5 del HCV es codificada por la región putativa NS5 del genoma del HCV. Un porcentaje importante de las personas infectadas por el virus de la hepatitis C presenta anticuerpos frente a la proteína NS5[3].

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El huésped de los tres antígenos recombinantes del HCV es *Saccaromyces cerevisiae* (levadura).

Principios del procedimiento

El test *Vitros* anti-HCV se realiza con el Kit de Reactivos *Vitros* anti-HCV y el calibrador *Vitros* anti-HCV en el sistema de Inmunodiagnóstico *Vitros*.

Se utiliza una técnica inmunométrica, que implica una reacción en dos fases. En la primera fase, el anticuerpo dirigido contra el HCV presente en la muestra se fija a los antígenos recombinantes del HCV del revestimiento de los pocillos. La muestra no fijada se elimina mediante lavado. En la segunda fase, el conjugado de anticuerpos (monoclonal de ratón anti-IgG humana) marcado con peroxidasa de rábano picante se une a las IgG humanas fijadas al pocillo durante la primera fase. El conjugado no fijado se elimina mediante lavados.

El conjugado HRP fijado se determina mediante una reacción luminiscente[47]. Se añade a los pocillos un reactivo que contiene sustratos luminogénicos (un derivado luminol y una sal perácido) y un agente de transferencia de electrones. La HRP en el conjugado fijado cataliza la oxidación del derivado de luminol y produce luz. El agente de transferencia de electrones (una acetanilida sustituida) incrementa el nivel de luz producido y prolonga su emisión. Las señales luminosas son leídas por el Sistema *Vitros*. La cantidad de conjugado de peroxidasa de rábano picante fijado es directamente proporcional a la concentración de anti-HCV presente.

Advertencias y precauciones

Para uso diagnóstico *In Vitro*

Advertencia - Material potencialmente infeccioso

- Deberá tratarse como si pudiera transmitir infección.
- Tanto la manipulación de las muestras y los componentes del test, como su utilización, almacenamiento y eliminación deberán realizarse de acuerdo con los procedimientos definidos en las directrices o reglamentos nacionales apropiados sobre seguridad de productos biopeligrosos (p. ej., la directiva M29 del NCCLS M29[55]).

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El calibrador *Vitros* anti-HCV contiene:
Plasma positivo a anticuerpos frente al HCV, obtenido de donantes que fueron analizados individualmente y que dieron un resultado negativo al antígeno de superficie de la hepatitis B, y a los anticuerpos contra el virus de la inmunodeficiencia humana (HIV 1+2), mediante métodos aprobados (inmunoensayos enzimáticos). El plasma positivo a los anticuerpos frente HCV ha sido tratado a fin de reducir la concentración de virus potencialmente infecciosos. Sin embargo, como ningún método de análisis puede descartar el riesgo de infección, debe manipularse como si pudiera transmitir infecciones.

Plasma negativo para anticuerpos frente al HCV, obtenido de donantes que fueron analizados individualmente y que resultaron negativos al antígeno de superficie de la hepatitis B, y a anticuerpos frente a HIV 1+2 y HCV, mediante métodos aprobados (inmunoensayos enzimáticos).

Deberá tenerse cuidado al manipular materiales de origen humano. Todos las muestras deberán considerarse como potencialmente infecciosas. Ninguno de los métodos actuales ofrece plenas garantías de ausencia del virus de la hepatitis B, del HCV, del HIV 1+2 o de cualquier otro agente infeccioso.

Advertencia: contiene ProClin 300 y 2-cloroacetamida

El reactivo conjugado contiene ProClin 300 y el reactivo del test contiene 2-cloroacetamida. R43: Posibilidad de sensibilización en contacto con la piel. R52/53: Nocivo para los organismos acuáticos, puede provocar a largo plazo efectos negativos en el medio ambiente acuático. S24: Evítese el contacto con la piel. S37: Úsense guantes adecuados.

ES

Materiales que se incluyen

1 kit de reactivos contiene:

- 100 pocillos recubiertos (antígenos recombinantes del virus de la hepatitis C derivados de levaduras; recubiertos con 0,41 µg/pocillo).
- 18,2 ml de reactivo del análisis (solución tampón con un agente antimicrobiano).
- 20,6 ml de reactivo conjugado (monoclonal de ratón anti-IgG humana, marcado con peroxidasa de rábano picante, 1,04 ng/pocillo) en solución tampón de suero fetal de ternero con un agente antimicrobiano.

Nota: Contiene albúmina sérica bovina y suero fetal de ternero.

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Materiales necesarios que no se incluyen

El Sistema de Inmunodiagnóstico *Vitros* y los siguientes productos de Inmunodiagnóstico *Vitros*: Calibrador anti-HCV (incluida la tarjeta de calibración de lote y la tarjeta de protocolo), reactivo de señal, solución de lavado universal, estuche de conservación del kit de reactivos (optativo) con desecante.

Preparación y conservación de los reactivos

El kit de reactivos se suministra listo para su uso. Conservar sin abrir entre 2-8 °C, no congelar. Los kits de reactivos abiertos deben utilizarse antes de 8 semanas desde el momento en que se cargan por primera vez en el sistema; no deben utilizarse después de la fecha de caducidad. Conservar los kits de reactivos abiertos a bordo del Sistema o a una temperatura de 2 - 8 °C en un estuche de conservación del kit de reactivos que contenga desecante seco.

El calibrador *Vitros* anti-HCV se suministra listo para su uso. Conservar sin abrir entre 2-8 °C. No utilizar después de la fecha de caducidad. Después de abrir, conservar hasta 13 semanas a una temperatura de 2 a 8 °C ó 13 semanas a -20 °C (sin aplicar más de un ciclo de congelación - descongelación).

Preparación del paciente

No se requiere ninguna preparación especial del paciente.

Recogida, preparación y conservación de las muestras

Pueden utilizarse muestras de suero o plasma (EDTA, heparina o citrato). Los resultados de las muestras de plasma con citrato serán proporcionalmente más bajas debido a la dilución por el anticoagulante líquido. Las muestras de sangre deben recogerse utilizando procedimientos estándar. Las muestras deberán separarse minuciosamente de todo el material celular. El incumplimiento de lo anterior podría ocasionar resultados falsamente elevados. Las muestras de suero y plasma pueden conservarse durante un período de hasta 7 días entre 2-8 °C o 4 semanas a -20°C. Debe evitarse congelar y descongelar las muestras repetidamente.

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En ocasiones, se ha comprobado que los recipientes especiales de recogida de muestras son perjudiciales para la integridad de ciertos analitos y pueden interferir con las tecnologías de algunos ensayos[26]. Debido a la variedad de recipientes disponibles para la recogida de muestras, Ortho-Clinical Diagnostics no puede proporcionar una declaración final acerca del rendimiento de sus reactivos con cada uno de estos recipientes. Se recomienda que cada usuario compruebe que el recipiente elegido se utiliza siguiendo las instrucciones del fabricante y que es compatible con el ensayo *Vitros*.

Control de calidad y notas de procedimiento

- Manipule el kit de reactivo con precaución, evitando: La formación de condensación en el envase; la formación de espuma en los reactivos; la agitación del envase.
- La calibración es específica de lote; los kits de reactivos y los calibradores están relacionados por un número de lote. Los kits pertenecientes al mismo lote pueden utilizar la misma calibración, que debe realizarse utilizando calibradores del mismo número de lote.
- Mezcle las muestras, los calibradores y los controles minuciosamente mediante inversión y espere a que alcancen 15 a 30 °C antes de su uso.
- Manipule las muestras, los calibradores y los controles en recipientes taponados para evitar la contaminación y la evaporación. Para evitar la evaporación, limite el tiempo que las muestras, los calibradores y los controles están cargados en el sistema *Vitros*. Si desea información adicional, consulte el Manual del Usuario del Sistema *Vitros*. Vuelva a poner a una temperatura de 2 a 8 °C tan pronto como sea posible después de su uso, o cargue sólo la cantidad suficiente para una única determinación. El calibrador puede dividirse en alícuotas y ponerse en recipientes distintos que se pueden identificar mediante las etiquetas de códigos de barras que se incluyen en el kit.
- El calibrador anti-HCV se procesa automáticamente por duplicado.

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- Compruebe el inventario regularmente para facilitar la gestión de los reactivos y compruebe que hay suficiente Reactivo de Señal *Vitros*, Solución de Lavado Universal *Vitros* y suficientes lotes de reactivos calibrados disponibles para el trabajo planificado. Cuando se realizan paneles de tests sobre una única muestra, compruebe que el volumen de la muestra es suficiente para el número de ensayos solicitados.
- La buena práctica de laboratorio requiere el procesamiento de controles para verificar el rendimiento del test. Existen dos Controles *Vitros* anti-HCV (anti-HCV negativo y anti-HCV positivo). Se recomienda procesar los controles al realizar una calibración y, posteriormente, por lo menos una vez cada 24 horas y después de realizar los procedimientos de servicio especificados (consultar el Manual del usuario del Sistema *Vitros*). Si los procedimientos de control de calidad vigentes en su laboratorio requieren un uso más frecuente de controles, siga dichos procedimientos Si desea información adicional, consulte el Manual del Usuario del Sistema *Vitros*.
- El nombre del análisis predeterminado que aparecerá en los informes de los pacientes es Anti-HCV. El nombre abreviado predeterminado que aparecerá en los menús de selección del análisis y en los informes del laboratorio es aHCV. Estos nombres predeterminados pueden reconfigurarse en caso necesario en la pantalla de Opciones y Configuración - Configurar Analitos.

Procedimiento

El test *Vitros* anti-HCV requiere 20 µl de muestra, calibrador o control para una determinación única. Esto no tiene en cuenta el volumen de llenado mínimo (volumen muerto) del envase elegido para la muestra.

El test *Vitros* anti-HCV debe calibrarse cada vez que se utiliza un lote de reactivo nuevo, y posteriormente en períodos de 28 días. Es posible que el test *Vitros* anti-HCV también requiera ser calibrado después de ciertos procedimientos de servicio o si los resultados del control de calidad están constantemente fuera de los límites aceptables.

Consulte las instrucciones detalladas sobre el funcionamiento del Sistema en el Manual del Usuario del Sistema Inmunodiagnóstico *Vitros*, Capítulos 4-7. En resumen:

1. Escanee la tarjeta de protocolo para cargar un nuevo protocolo de test en el Sistema. A continuación aparece el botón del test en la pantalla de Programación de Muestras. Escanee la tarjeta de calibración del lote de cada lote de reactivo nuevo para introducir la información de calibración y caducidad específica del lote.
2. Abra la bolsa metalizada y saque el kit del reactivo. Cargue el kit en la estación de autocarga, o utilice el botón Descargar/Cargar en la pantalla de Gestión de Reactivos - Ver por Reactivos. **Nota:** No use el producto si está dañado o si no está cerrado herméticamente.
3. Cargue las muestras en el Rotor Universal de Muestras, utilizando adaptadores cuando sea necesario (las muestras pueden tener códigos de barras si se desea). Coloque una punta de pipeta junto a cada muestra y cargue el rotor en el Sistema. Defina los programas de la muestra utilizando la pantalla de Programación de Muestras. Inicie el procesamiento de muestras; todos los pasos del proceso se llevarán a cabo automáticamente.
4. Procese el calibrador de la misma manera que las muestras (cargando suficiente cantidad para la determinación automática por duplicado). La calibración no tiene que programarse si se utilizan etiquetas de códigos de barras; la calibración se iniciará automáticamente.

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Resultados

Los resultados se calculan como una señal normalizada, respecto a un valor de corte. Durante el proceso de calibración se utiliza un parámetro específico de lote, codificado en la tarjeta de calibración, para determinar un valor de corte válido para el Sistema. Para más detalles sobre la calibración, consulte las Instrucciones de uso que se suministra con el calibrador anti-HCV. El Sistema *Vitros* calcula automáticamente los resultados.

$$\text{Resultado} = \frac{\text{Señal de la muestra analizada}}{\text{Valor de corte}}$$

Un resultado ≥1,00 indica una muestra reactiva y la posible presencia de anti-HCV.
Un resultado <0,90 indica una muestra no reactiva, negativa para anti-HCV.
Un resultado ≥0,90 y <1,00 indica una muestra dudosa.

Control de Calidad

- Los resultados de la calibración se valoran frente a dos parámetros de calidad detallados en Ver sección Parámetros de Cal, a los que se accede a través de la pantalla de Opciones y Configuración - Revisión/Calibración del Usuario. Si algún parámetro de calidad definido no cumple los requisitos exigidos, esta información quedará codificada en el informe de calibración. Para las acciones a tomar tras una calibración fallida, consulte el Manual del Usuario, Capítulo 8, Revisión de Resultados.
- Los valores de la muestra del paciente se indicarán como "Negativa", "Dudosa" o "Reactiva". Los valores de control aparecerán con un indicador cuando tengan ≥2 DS con respecto la media inicial definida.
- No hay un parámetro único que pueda describir completamente la calidad de la calibración. Para determinar la validez de la calibración, se utilizará el informe de calibración junto con los valores de control.
- Si los resultados de control se sitúan fuera de unos límites aceptables, investigue la causa antes de notificar los resultados.

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Limitaciones del procedimiento

- Los resultados de este o cualquier otro kit de diagnóstico deberán utilizarse e interpretarse únicamente en el contexto del cuadro clínico global. Un resultado negativo no excluye la posibilidad de exposición o infección por el HCV. Es posible que en algunas de las fases de la infección y en algunas patologías clínicas los anticuerpos frente a HCV no sean detectables[7].
- Los anticuerpos heterófilos en las muestras de suero o plasma pueden causar interferencias en los inmunoensayos[8]. Estos anticuerpos pueden estar presentes en las muestras sanguíneas de pacientes habitualmente expuestos a animales o que han sido tratados con productos basados en suero de animales. Los resultados que no son coherentes con las observaciones clínicas indican la necesidad de realizar tests adicionales.
- Las muestras que contienen trioleína (<33,9 mmol/l), hemoglobina (<5 g/l) o bilirrubina (<0,342 mmol/l) no interfieren con la interpretación clínica de estos resultados. No utilizar muestras turbias.

Interpretación de los resultados

Toda muestra dudosa o reactiva debe volver a analizarse por duplicado para verificar su estado. Antes de volver a ensayarlas, la muestra deberá centrifugarse para garantizar que está libre de células, restos de células o fibrina. Si, al repetir los ensayos, los resultados son <0,90 en ambas repeticiones, se considerará que la muestra es negativa. Si el resultado de una de las repeticiones es ≥0,90 se volverá a ensayar la muestra con pruebas complementarias para confirmar el resultado. Una muestra repetidamente reactiva, confirmada por pruebas suplementarias, deberá considerarse positiva para anti-HCV. En caso de resultados repetidamente dudosos, se recomienda analizar muestras de seguimiento.

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Características de rendimiento

Calibración y trazabilidad de la calibración
La trazabilidad de la calibración del test *Vitros* anti-HCV es posible mediante un calibrador de referencia interno que tiene un valor asignado para optimizar la sensibilidad clínica y la especificidad.

Precisión
La precisión se evaluó siguiendo el protocolo EP5-T2 del Comité Nacional de Normas para Laboratorios Clínicos[9]. Se ensayaron dos repeticiones de cada uno de las 4 muestras de panel una sola vez al día en al menos 20 días diferentes. El experimento se realizó utilizando 2 lotes de reactivo en Sistemas diferentes. Los datos que se presentan son representativos del rendimiento del producto.

Tabla 1: Precisión

Resultado	Intra-serie		Intra-calibración		Intra-laboratorio	
representativa	DS*	CV(%)*	DS*	CV(%)*	DS*	CV(%)*
0,14	0,00336	2,4	0,00835	4,9	0,00945	6,9
2,04	0,044	2,1	0,132	6,4	0,134	6,6
5,74	0,132	2,4	0,339	5,8	0,354	6,2
14,9	0,187	1,2	0,454	3,1	0,576	3,9

* Media cuadrática (MC)

Intra-serie: Promedio de la precisión entre duplicados de todas las series.
Intra-calibración Precisión total, con componentes ponderados de la variación intra-serie, e inter-días.
Intra-laboratorio: Medida del efecto de recalibración sobre la precisión total, calculada intra-lote de reactivo, utilizando datos de 4 calibraciones.

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Sensibilidad
Utilizando *Vitros* anti-HCV se analizaron 435 muestras de pacientes anteriormente determinadas como positivas mediante un análisis inmunoblot para HCV. La sensibilidad observada en esta población de muestras con el test *Vitros* anti-HCV fue del 100% (435/435).

Adicionalmente se ensayaron otros 29 paneles de seroconversión comerciales. Al comparar los resultados de *Vitros* anti-HCV con los de otro test presente en el mercado, se demostró una sensibilidad de seroconversión equivalente o superior en 29/29 grupos.

Especificidad
Las muestras de 5.374 donantes de sangre presuntamente sanos y 393 muestras clínicas se analizaron con el test *Vitros* anti-HCV y con otro test comercial.

Muestras	Número de muestras	Inicialmente reactivas	Repetidamente reactivas	Confirmadas positivas
Donante	5.374	14	13	0
Clínicas	393	1	1	0

La especificidad calculada del test *Vitros* anti-HCV para la población de donantes fue del 99,76% (5.361/5.374), considerando las muestras repetidamente reactivas. La especificidad calculada del test *Vitros* anti-HCV para la población clínica fue del 99,75% (392/393), considerando las muestras repetidamente reactivas.

Adicionalmente, con el test *Vitros* Anti-HCV se ensayaron 161 muestras de los siguientes grupos de potencial reactividad cruzada: positivos al citomegalovirus, positivos al virus de Epstein-Barr, positivos al HIV, pacientes con enfermedades hepáticas no víricas, otras enfermedades hepáticas víricas (p. ej., HBV, HAV), lupus eritematoso sistémico, positivos al factor reumatoide, recién vacunados (p. ej., contra la gripe), muestras reactivas a levaduras. En ninguna de estas categorías se obtuvieron resultados falsos reactivos en el test *Vitros* anti-HCV.

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Grupo de trabajo ADM: Resumen de la evaluación

El grupo de trabajo ADM evaluó el test *Vitros* anti-HCV. Se analizaron 450 muestras procedentes de 246 pacientes infectados por diferentes genotipos del virus de la hepatitis C y 50 muestras del HCV SFTS, incluidas 27 muestras ARN reactivas. El test *Vitros* anti-HCV demostró una buena sensibilidad para la detección de la seroconversión precoz y para el cribado de otras categorías de muestras reactivas, incluidas las que mostraban reactividad aislada y las procedentes de portadores crónicos.

Se analizó a una población de 2018 muestras de donantes de sangre con el test *Vitros* anti-HCV. Ocho muestras produjeron resultados repetidamente reactivos, una de los cuales se confirmó como reactiva y 6 resultaron negativas mediante otros análisis. El resultado de la muestra restante no se pudo interpretar (NS3 indeterminado, PCR negativo). La especificidad del test *Vitros* anti-HCV fue de aproximadamente el 99,70%.

Declaración de licencia

Los antígenos recombinantes del HCV utilizados en el test *Vitros* anti-HCV han sido preparados bajo licencia estadounidense por Chiron Corporation, según un acuerdo de fabricación compartido.

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Vitros Immunodiagnostic Products
Anti-HCV-reagenspakke

Tilsigtet anvendelse

Til kvalitativ *in vitro*-påvisning af antistoffer mod hepatitis C-virus (anti-HCV) i humant serum og plasma (EDTA, heparin eller citrat).

Sammendrag og forklaring af testen

Hepatitis C-virus (HCV) vides nu at være årsagen til de fleste, hvis ikke alle, tilfælde af blodoverført non-A, non-B-hepatitis (NANBH). Studier over hele verden viser, at HCV overføres via kontamineret blod og kontaminerede blodprodukter gennem blodtransfusioner eller andre former for tæt personlig kontakt. Tilstedeværelse af anti-HCV indikerer, at en person kan være smittet med HCV og kan være i stand til at overføre HCV-infektion[1].

Der anvendes tre rekombinante hepatitis C-virus-kodede antigener i *Vitros* anti-HCV-analysen. De tre rekombinante antigener er c22-3, c200 og NS-5. Det rekombinante protein c22-3 kodes af HCV-genomets formodede coreregion. Det rekombinante HCV-protein c200 kodes af HCV-genomets formodede NS3- og NS4-region. Proteinet c200 indeholder proteinsekvensen c33c, som genetisk er forbundet til proteinsekvensen c100-3. Studier har vist, at antistoffer, som udvikles efter infektion med HCV, ofte er reaktive med c22-3 og eller c33c[2]. Det rekombinante HCV-protein NS5 kodes af HCV-genomets formodede NS5-region. En væsentlig del af de personer, der inficeres med HCV, udvikler antistoffer mod NS5[3].

Værtsorganismen for alle tre rekombinante HCV-antigener er *S. cerevisiae* (gær).

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Principper for proceduren

Vitros anti-HCV-analysen udføres ved brug af *Vitros* anti-HCV-reagenspakken og *Vitros* Immunodiagnostic Products anti-HCV-kalibratoren i *Vitros* Immunodiagnostic System.

Der anvendes en immunometrisk teknik, hvilket indebærer en totrinsreaktion. På det første trin binder det HCV-antistof, som er tilstede i prøven, sig til rekombinante HCV-antigener, som er coatet på brøndene. Ubundet prøve fjernes ved vask. På det andet trin binder peberrodsperoxidase (horseradish peroxidase (HRP))-mærket antistofkonjugat (murint, monoklonalt anti-humant IgG) sig til ethvert humant IgG, der er blevet fanget på brønden på det første trin. Ubundet konjugat fjernes ved vask.

Det bundne HRP-konjugat måles ved hjælp af en luminescerende reaktion[4]. Brøndene tilsættes et reagens, der indeholder luminogene substrater (et luminolderivat og et persyresalt), og et elektronoverførselsreagens. HRP i det bundne konjugat katalyserer oxideringen af luminolderivatet, hvorved der frembringes lys.
Elektronoverførselsreagenset (et substitueret acetanilid) forøger styrken af det frembragte lys og forlænger dets emission. Lyssignalerne aflæses af *Vitros*-systemet. Mængden af bundet HRP-konjugat er direkte proportional med koncentrationen af det tilstedeværende anti-HCV.

Advarsler og forholdsregler

Kun til *in vitro*-diagnostisk brug.

Advarsel – potentielt smittefarligt materiale

- Skal behandles, som potentiel smittefarlig.
- Håndtering af prøver og analysekomponenter, brug og opbevaring af dem samt bortskaffelse af fast og flydende affald skal ske i overensstemmelse med de procedurer, der defineres af de relevante nationale sikkerhedsretningslinjer eller -bestemmelser for miljø- og sundhedsskadeligt materiale (f.eks. NCCLS Guideline M29[5]).

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Vitros anti-HCV-kalibratoren indeholder:
HCV-antistofpositivt plasma indsamlet fra donorer, som er blevet testet individuelt, og som er fundet negative for hepatitis B-overfladeantigen og for antistoffer mod humant immundefekt virus (HIV 1 + 2) ved brug af godkendte metoder (enzymimmunanalyser). Det HCV-antistofpositive plasma er blevet behandlet for at reducere titeren af potentielt smittefarligt virus. Men da ingen testmetode kan udelukke risikoen for potentiel infektion, skal det behandles som potentielt smittefarligt.

HCV-antistofnegativt plasma indhentet fra donorer, som er blevet testet individuelt, og som er blevet fundet negative for hepatitis B-overfladeantigen og for antistoffer mod HCV og HIV 1 + 2 ved brug af godkendte metoder (enzymimmunanalyser).

Der skal udvises forsigtighed ved håndtering af materiale, der stammer fra mennesker. Alle prøver skal betragtes som potentielt smittefarlige. Ingen testmetode kan give fuldstændig sikkerhed for, at der ikke er hepatitis B-virus, HCV, HIV 1+2 eller andre smitstoffer til stede.

Advarsel – indeholder ProClin 300 og 2-chloracetamid
Konjugatreagenset indeholder ProClin 300, og analysereagenset indeholder 2-chloracetamid. R43: Kan give allergi ved kontakt med huden. R52/53: Skadeligt for organismer, der lever i vand, kan forårsage uønskede langtidsvirkninger i vandmiljøet. S24: Undgå kontakt med huden. S37: Brug egnede beskyttelseshandsker under arbejdet.

Leverede materialer

1 reagenspakke indeholdende:
- 100 coatede brønde (rekombinante hepatitis C-virusantigener udledt af gær; coatet med 0,41 µg/brønd).
- 18,2 ml analysereagens – (buffer med antimikrobielt middel).
- 20,6 ml konjugatreagens – (HRP-murint monoklonalt anti-humant IgG, 1,04 ng/brønd) i kalvefosterserum med buffer og antimikrobielt middel.

Bemærk: Indeholder bovint serumalbumin og kalvefosterserum.

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Materialer, der er nødvendige, men som ikke leveres

Vitros immundiagnostisk system og de følgende *Vitros* immundiagnostics products: Anti-HCV-kalibrator (inklusive lotspecifikt kalibreringskort og protokolkort), signalreagens, universal vaskereagens, opbevaringsboks til reagenspakker (valgfri) med tørremiddel.

Klargøring og opbevaring af reagenser

Reagenspakken leveres klar til brug. Skal opbevares uåbnet ved 2-8°C, må ikke nedfryses. Brug åbnede reagenspakker inden for 8 uger fra første isætning i et system. Må ikke anvendes efter udløbsdatoen. Åbnede reagenspakker skal opbevares i systemet eller ved 2-8°C i en lukket opbevaringsboks til reagenspakker indeholdende tørt tørremiddel.

Anti-HCV-kalibratoren leveres klar til brug. Skal opbevares uåbnet ved 2-8 °C. Må ikke anvendes efter udløbsdatoen. Opbevares efter åbning i op til 13 uger ved 2-8°C eller 13 uger ved -20°C (med højst 1 nedfrysnings-/optøningscyklus).

Forberedelse af patient

Speciel forberedelse af patienter er ikke nødvendig.

Indsamling, klargøring og opbevaring af prøver

Der kan anvendes serum- eller plasmaprøver (EDTA, heparin eller citrat). Resultater af citrat-plasmaprøver vil være forholdsvist lavere som følge af den fortynding, der sker med den flydende antikoagulans. Blodprøver skal indsamles ved brug af standardprocedurer. Prøverne skal separeres omhyggeligt fra alt cellemateriale. Hvis dette undlades, kan det medføre et falsk forhøjet resultat. Serum- og plasmaprøver kan opbevares i op til 7 dage ved 2-8°C eller 4 uger ved -20°C. Undgå at nedfryse og optø mere end en gang.

Nogle glastyper til prøvetagning er rapporteret at påvirke visse analytters integritet, og at de kunne gribe forstyrrende ind i nogle metodeteknologier[6]. På grund af de mange forskellige glastyper, der findes, er Ortho-Clinical Diagnostics ikke i stand til at give en definitiv udtalelse om, hvordan firmaets produkter opfører sig sammen med disse. Det anbefales, at hver enkelt bruger sikrer, at den valgte glastype anvendes i overensstemmelse med producentens instrukser, og at den er kompatibel med den pågældende *Vitros*-analyse.

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Kvalitetskontrol og proceduremæssige bemærkninger

- Håndter reagenspakken med forsigtighed, og undgå det følgende: at der dannes kondens på pakken; at få reagenserne til at skumme; at ryste pakken.
- Kalibrering er lotspecifik. Reagenspakker og kalibratorer kædes sammen af et lotnummer. Reagenspakker fra samme lot kan anvende den samme kalibrering. Denne skal udføres ved brug af en kalibrator med samme lotnummer.
- Bland prøverne, kalibratorerne og kontrollerne grundigt ved at vende dem, og bring dem op på 15-30°C før brug.
- Håndter prøver, kalibratorer og kontroller i tilproppede prøverør for at undgå kontaminering og fordampning. For at undgå fordampning skal den tid, hvor prøver, kalibratorer og kontroller befinder sig i *Vitros*-systemet, begrænses. Der henvises til *Vitros*-systemets brugervejledning for yderligere information. Returner til 2-8 °C så hurtigt som muligt efter brug, eller især kun tilstrækkeligt til en enkelt anvendelse. Kalibratoren kan opdeles i flere prøverør, som kan stregkodes med de leverede mærkater.
- Anti-HCV-kalibratoren behandles automatisk som dobbeltbestemmelse.
- Tjek reagensbeholdningen regelmæssigt for at understøtte styringen af reagenser og sikre, at der er tilstrækkeligt *Vitros*-signalreagens, *Vitros*-universal vaskereagens og kalibrerede reagenslot til rådighed til det planlagte arbejde. Ved udførelse af analysepaneler på en enkelt prøve skal det kontrolleres, at prøvevolumenet er tilstrækkeligt til de bestilte analyser.
- God laboratoriepraksis kræver, at der udføres kontroller for at verificere analysekvaliteten. Der er 2 *Vitros* anti-HCV-kontroller (anti-HCV-negativ og anti-HCV-positiv). Det anbefales, at der udføres kontroller, når der er foretaget en kalibrering og derefter mindst en gang hver 24. time, samt efter at specificerede vedligeholdelsesprocedurer er udført (der henvises til *Vitros*-systemets brugervejledning). Hvis laboratoriets kvalitetskontrolprocedurer kræver hyppigere brug af kontroller, skal disse procedurer følges. Der henvises til *Vitros*-systemets brugervejledning for mere detaljeret information.

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- Det standardanalysenavn, som vil blive vist på patientrapporter, er anti-HCV. Det korte standardnavn, som vil blive vist på menuerne til valg af analyse og på laboratorierapporterne, er aHCV. Disse standarder kan om nødvendigt rekonfigureres på skærmen Options & Configurations - Configure Analytes.

Procedure

Vitros anti-HCV-analysen kræver 20 µl prøve, kalibrator eller kontrol til en enkelt bestemmelse. Dette tager ikke hensyn til det valgte prøverørs minimumspåfyldningsvolumen.

Vitros anti-HCV-analysen skal kalibreres hver gang, der anvendes et nyt reagenslot, og efterfølgende med intervaller på 28 dage. Det kan også være nødvendigt at kalibrere *Vitros* anti-HCV-analysen efter udførelse af visse vedligeholdelsesprocedurer; eller hvis resultaterne af kvalitetskontrollen konsekvent ligger uden for det acceptable område.

Angående detaljeret vejledning i betjening af systemet henvises der til brugervejledningen til *Vitros* immundiagnostisk system, kapitlerne 4-7. Sammendrag:

1. Indscan protokolkortet for at indlægge en ny analyseprotokol i systemet. Analyseknappen vises derefter på skærmen Sample Programming. Indscan det lotspecifikke kalibreringskortet for hvert nyt reagenslot for at indlæse lotspecifikke kalibrerings- og udløbsinformation.
2. Åbn folieposen, og tag reagenspakken ud. Sæt pakken i den automatiske indføringsstation, eller brug knappen Unload/Load på skærmen Reagent Management - View by Reagent. **Bemærk:** Et beskadiget produkt eller et produkt, der ikke er fuldstændigt forseglet, må ikke anvendes.
3. Sæt prøverne i universalprøvebakker ved brug af adaptere, når det er nødvendigt (prøver kan påsættes stregkoder, hvis det ønskes). Anbring en engangsspids ud for hver enkelt prøve, og sæt bakkerne i systemet. Definer prøveprogrammer ved brug af skærmen Sample Programming. Start prøveanalyseringen. Alle prøvebehandlingstrin udføres automatisk.
4. Kalibratoren behandles på samme måde som prøver (idet der påfyldes tilstrækkeligt til den automatiske dobbeltbestemmelse). Kalibreringen behøver ikke at blive programmeret, hvis der anvendes stregkodemærkater. Kalibreringen påbegyndes automatisk.

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Resultater

Resultaterne beregnes som et normaliseret signal i forhold til en grænseværdi. Under kalibreringsprocessen anvendes et lotspecifikt parameter, der er indkodet på det lotspecifikke kalibreringskort, til at bestemme en gyldig, lagret cut-off værdi for systemet. For yderligere oplysninger om kalibrering henvises der til brugsanvisningen, der leveres sammen med anti-HCV-kalibratoren. Resultaterne beregnes automatisk af *Vitros*-systemet.

$$\text{Resultat} = \frac{\text{Signal for testprøve}}{\text{Cut-off værdi}}$$

Et resultat på ≥1,00 indikerer en reaktiv prøve og mulig tilstedeværelse af anti-HCV.
Et resultat på <0,90 indikerer en ikke-reaktiv prøve, der er negativ for anti-HCV.
Et resultat på ≥0,90 og <1,00 indikerer en prøve med gråzoneværdi.

Kvalitetskontrol

- Kalibreringsresultaterne vurderes over for to kvalitetsparametre som er specificerede i View Cal Parameters, som man får adgang til via skærmen Options & Configuration - Review/User Calibrations. Hvis et af de definerede kvalitetsparameterområder ikke opnås, vil det blive markeret med en kode på kalibreringsrapporten. For hvad der skal gøres efter en mislykket kalibrering henvises der til brugervejledningen, kapitel 8, Gennemgang af resultater.
- Patientprøveværdierne markeres med 'Negativ', 'Gråzoneværdi' eller 'Reaktiv'. Kontrolværdierne markeres, når de afviger ≥2 SD i forhold til den definerede middelværdi for basislinje.
- Kalibreringskvaliteten kan ikke beskrives fuldt ud af et enkelt parameter. For at bestemme kalibreringens validitet skal kalibreringsrapporten anvendes sammen med kontrolværdier.
- Hvis kontrolresultaterne falder uden for det acceptable område, skal årsagen undersøges, før det afgøres, om patientresultater kan rapporteres.

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Procedurens begrænsninger

- Resultaterne fra dette eller ethvert andet diagnosticeringskit bør kun anvendes og tolkes i sammenhæng med det samlede kliniske billede. Et negativt testresultat udelukker ikke muligheden af eksponering til HCV eller infektion dermed. HCV-antistoffer kan være upåviselige på nogle infektionsstadier og ved visse kliniske tilstande[7].
- Heterofile antistoffer i serum- eller plasmaprøver kan forårsage interferens ved immunanalyser[8]. Disse antistoffer kan være til stede i blodprøver fra personer, der regelmæssigt kommer i kontakt med dyr, eller som har været behandlet med serumprodukter fra dyr. Resultater, som er uforenelige med kliniske observationer, viser, at der er behov for yderligere testning.
- Prøver, der indeholder triolein (<33,9 mmol/l), hæmoglobin (<5 g/l) eller bilirubin (<0,342 mmol/l), interfererer ikke med den kliniske tolkning af disse resultater. Uklare prøver må ikke anvendes.

Tolkning af resultater

En prøve, der konstateres at have gråzoneværdi eller at være reaktiv, skal dobbelttestes igen, for at dens status kan verificeres. Før prøven testes igen, skal den centrifugeres for at sikre, at den er fri for celler, cellulært débris eller fibrin. Hvis resultaterne ved fornyet testning er <0,90 for begge replikater, skal prøven anses for negativ. Hvis et af de to fornyede dobbelttestresultater er ≥0,90, skal prøven testes ved hjælp af supplerende test for at bekræfte resultatet. En prøve, der gentagne gange er reaktiv bekræftet ved supplerende test, skal anses for positiv for anti-HCV. Hvis resultaterne gentagne gange er gråzoneværdier, anbefales analysering af opfølgningsprøver.

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Funktionskarakteristika

Kalibrering og sporbarhed af kalibrering
Kalibreringen af *Vitros* anti-HCV-analysen kan spores til en intern referencekalibrator, som er blevet tildelt værdier for at optimere kvaliteten af den kliniske følsomhed og specificitet.

Præcision
Præcisionen er blevet vurderet i overensstemmelse med protokollen fra "National Committee for Clinical Laboratory Standards", EP5-T2[9]. To replikater, der hver består af 4 panelprøver, blev analyseret en enkelt gang pr. dag på mindst 20 forskellige dage. Forsøget blev udført ved brug af 2 reagenslot i forskellige systemer. De præsenterede data er repræsentative for produktets funktion.

Tabel 1: Præcision

Repræsentativ resultat	Inden for kørsel		Inden for kalibrering		Inden for lab.	
	SD*	CV(%)*	SD*	CV(%)*	SD*	CV(%)*
0,14	0,00336	2,4	0,00835	4,9	0,00945	6,9
2,04	0,044	2,1	0,132	6,4	0,134	6,6
5,74	0,132	2,4	0,339	5,8	0,354	6,2
14,9	0,187	1,2	0,454	3,1	0,576	3,9

* Root Mean Square (RMS) (gennemsnitlig kvadratrod)

Inden for kørsel: Gennemsnitlig præcision mellem dobbeltbestemmelser på tværs af alle kørsler.
Inden for kalibrering: Total præcision med vægtede komponenter af variation inden for kørsel og variation mellem dage.
Inden for lab.: Et udtryk for virkningen af rekalibrering på den totale præcision beregnet inden for et reagenslot ved brug af data fra 4 kalibreringer.

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Følsomhed

435 patientprøver, der tidligere var bestemt som positive ved hjælp af en HCV-immunoblotanalyse, blev testet med *Vitros* anti-HCV-analysen. Følsomheden for denne population af prøver blev ved *Vitros* anti-HCV-analysen beregnet som 100 % (435/435).

Derudover blev der testet 29 kommercielt tilgængelige serokonversionspaneler. *Vitros* anti-HCV-analysen viste ækvivalent eller større serokonversionsfølsomhed for 29/29 paneler ved sammenligning med de publicerede resultater fra anden kommercielt tilgængelig analyse.

Specificitet

Prøver fra 5.374 antageligt raske bloddonorer og 393 kliniske prøver er blevet testet med *Vitros* anti-HCV-analysen og anden kommercielt tilgængelig analyse.

Prøver	Antal testprøver	Initialt reaktiv	Gentagne gange reaktiv	Bekræftet positiv
Donor	5.374	14	13	0
Klinisk	393	1	1	0

Specificiteten af *Vitros* anti-HCV-analysen er for donorpopulationen blevet beregnet som 99,76 % (5.361/5.374) på basis af gentagne reaktive prøver. Specificiteten af *Vitros* anti-HCV-analysen er for den kliniske populationen blevet beregnet som 99,75 % (392/393) på basis af gentagne reaktive prøver.

Yderligere 161 prøver fra de følgende potentielt krydsreagerende undergrupper er blevet testede med *Vitros* anti-HCV-analysen: CMV-positive, EBV-positive, HIV-positive, patienter med ikke-viral leversygdom, andre virale leversygdomme (f.eks. HBV, HAV), SLE, rheumafaktor-positive, personer, der nyligt var vaccinerede (f.eks. influenza), gærreaktive prøver. Af disse kategorier gav ingen falsk reaktive resultater ved *Vitros* anti-HCV-analysen.

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ADM-arbejdsgruppen: Resume af vurdering

Vitros anti-HCV-analysen er blevet vurderet af ADM-arbejdsgruppen. 450 prøver fra 246 patienter inficerede med forskellige genotyper af hepatitis C-virus og 50 prøver fra HCV SFTS-panelet, inklusive 27 RNA-reaktive prøver, blev testet. *Vitros* anti-HCV-analysen viste god følsomhed for påvisningen af tidlig serokonversion og for screeningen af andre kategorier af reaktive prøver, inklusive prøver, der viste isoleret reaktivitet, og prøver fra kroniske bærere.

En population på 2018 bloddonorprøver blev testet med *Vitros* anti-HCV-analysen. Otte prøver gav gentagne reaktive resultater, hvoraf 1 blev bekræftet som reaktiv og 6 blev fundet negative ved hjælp af andre analyser. Resultatet af resten af prøven kunne ikke tolkes (ubestemmelig NS3, PCR-negativ). *Vitros* anti-HCV-analysens specificitet var ca. 99,70 %.

Licenserklæring

De rekombinante HCV-antigener, der anvendes ved *Vitros* anti-HCV-analysen, er af Chiron Corporation præparerede under amerikansk (USA) licens i henhold til en fælles fabrikationsaftale.

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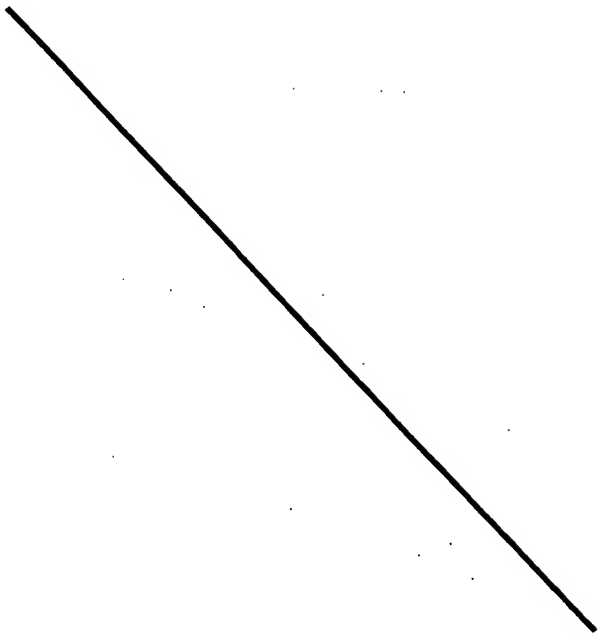

Vitros Immunodiagnostic Products
Anti-HCV reagenspack

Avsedd användning

För kvalitativ detektion *in vitro* av antikroppar mot hepatit C-virus (anti-HCV) i humant serum och plasma (EDTA, heparin eller citrat).

Sammanfattning och förklaring av analys

Numera vet man att hepatit C-viruset (HCV) är orsaken till största delen av, om inte all, blodburen non-A, non-B-hepatit (NANBH). Studier över hela världen visar att HCV överförs via kontaminerat blod och kontaminerade blodprodukter, via blodtransfusioner eller via andra nära, personliga kontakter. Förekomsten av anti-HCV visar att en individ kan ha infekterats med HCV och eventuellt kan överföra HCV-infektion[1].

Tre rekombinanta hepatit C-viruskodade antigener används i *Vitros* anti-HCV-analys. De tre rekombinanta antigenerna är c22-3, c200 och NS-5. Det rekombinanta proteinet c22-3 kodas med det förmodade kärnområdet för HCV-genomet. HCV-rekombinant protein c200 kodas med de förmodade NS3- och NS4-områdena för HCV-genomet. c200-proteinet innehåller c33c-proteinsekvensen som är genetiskt kopplad till c100-3-proteinsekvensen. Studier har visat att antikroppar som utvecklas efter infektion med HCV ofta är reaktiva mot c22-3 och eller c33c[2]. HCV-rekombinant protein NS5 kodas med det förmodade NS5-området för HCV-genomet. En signifikant andel av de människor som är infekterade med HCV utvecklar antikroppar mot NS5[3].

Värdorganismen för alla tre HCV-rekombinanta antigener är *S. cerevisiae* (jäst).

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Metodprinciper

Vitros anti-HCV-analys utförs med användning av *Vitros* anti-HCV reagenspack och *Vitros* immunodiagnostiska produkter anti-HCV-kalibrator i *Vitros* immunodiagnostiska system.

En immunometrisk teknik används, i vilken ingår en tvåstegsreaktion. I det första steget binds HCV-antikroppar som förekommer i provet till HCV-rekombinanta antigener som brunnarna är coatade med. Obundet prov tvättas bort. I det andra steget binds pepparotsperoxidas(HRP)-märkt antikroppskonjugat (musmonoklonalt anti-humant IgG) till det humana IgG som fångats in på brunnen i det första steget. Obundet konjugat tvättas bort.

Det bundna HRP-konjugatet mäts genom en luminiscensreaktion[4]. Ett reagens som innehåller luminogena substrat (ett luminolderivat och ett peracidsalt) och ett elektronöverföringsmedel, tillsätts i brunnarna. HRP i det bundna konjugatet katalyserar oxideringen av luminolderivatet, vilket producerar ljus. Elektronöverföringsmedlet (en ersatt acetanilid) ökar ljusstyrkan och förlänger tiden för emissionen. *Vitros*-systemet mäter ljussignalerna. Mängden bundet HRP-konjugat är direkt proportionell mot den närvarande anti-HCV-koncentrationen.

Varningar och försiktighetsåtgärder

Endast för diagnostisk användning *in vitro*

Varning - potentiellt smittsamt material

- Hanteras som om de kan överföra smitta.
- Hantering av prov och analyskomponenter, deras användning och förvaring och kasseringen av fast och flytande avfall skall utföras i enlighet med de procedurer som fastställs i tillämpliga nationella säkerhetsriktlinjer eller föreskrifter för biologiskt riskavfall (t.ex. NCCLS Guideline M29[5]).

Vitros anti-HCV-kalibrator innehåller:
HCV-antikroppspositiv plasma som erhållits från givare som testats individuellt och befunnits vara negativa beträffande hepatit B ytantigen, och beträffande antikroppar mot humant immunbristvirus (HIV 1+2), med användning av vedertagna metoder (enzymimmunanalyser). Den HCV-antikroppspositiva plasman har behandlats för att reducera titern för potentiellt smittsamt virus. Eftersom ingen analysmetod helt kan utesluta risken för potentiell infektion, skall den dock hanteras som om den kan överföra smitta.

HCV-antikroppsnegativ plasma som erhållits från givare som testats individuellt och befunnits vara negativa beträffande hepatit B ytantigen, och beträffande antikroppar mot HCV och HIV 1+2, med användning av vedertagna metoder (enzymimmunanalyser).

Var försiktig vid hantering av material med humant ursprung. Alla prov skall betraktas som potentiellt smittsamma. Ingen analysmetod kan ge total garanti för att hepatit B-virus, HCV, HIV 1+2 eller andra smittsamma ämnen inte förekommer.

Varning – Innehåller ProClin 300 och 2-kloracetamid

Konjugatreagenset innehåller ProClin 300 och analysreagenset innehåller 2-kloracetamid. R43: Kan ge allergi vid hudkontakt. R52/53: Skadligt för vattenlevande organismer, kan orsaka skadliga långtidseffekter i vattenmiljön. S24: Undvik kontakt med huden. S37: Använd lämpliga skyddshandskar.

Tillhandahållet material

1 st. reagenspack innehåller:
- 100 coatade brunnar (hepatit C-virusrekombinanta antigener utvunna från jäst; coatade med 0,41 µg/brunn).
- 18,2 mL analysreagens - (buffert med antimikrobiellt medel).
- 20,6 mL konjugatreagens - (HRP-musmonoklonalt anti-humant IgG, 1,04 ng/brunn) i buffrat fetalt serum från kalv med antimikrobiellt medel.

Obs! Innehåller bovint serumalbumin och fetalt serum från kalv.

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Material som krävs, ej tillhandahållet

Vitros immunodiagnostiksystem och följande *Vitros* immunodiagnostikprodukter: Anti-HCV-kalibrator (inklusive lot-kalibrerings- och protokollkort), signalreagens, tvättreagens, förvaringsbox för reagenspack (tillval) med torkmedel.

Reagensberedning och -förvaring

Reagenspack levereras klart att använda. Förvara oöppnat vid 2-8°C, får ej frysas. Använd öppnade reagenspack inom 8 veckor efter att de laddats första gången i ett system; använd ej efter utgångsdatum. Förvara öppnade reagenspack i systemet, eller vid 2-8°C i en förseglad förvaringsbox för reagenspack med torkmedel.

Anti-HCV-kalibratorn levereras klar att använda. Förvaras oöppnad vid 2-8°C. Använd ej efter utgångsdatum. Lagras efter öppnande i upp till 13 veckor i 2–8°C eller 13 veckor i -20°C (med högst en 1 infrysnings-upptiningscykel).

Patientförberedelse

Det krävs ingen särskild patientförberedelse.

Provtagning, förberedelse och förvaring av prov

Serum- eller plasmaprov (EDTA, heparin eller citrat) kan användas. Resultat från citratplasmaprov kommer att vara proportionellt lägre på grund av spädning med den vätskeformiga antikoagulanten. Utför provtagning på sedvanligt sätt. Prov skall noga separeras från allt cellmaterial. Görs inte det, kan följden bli ett falskt förhöjt resultat. Serum- och plasmaprov kan förvaras i upp till 7 dagar vid 2–8°C eller 4 veckor vid -20°C. Undvik upprepad infrysning och upptining.

Anordningar för provinsamling har ibland rapporterats vara skadliga för vissa analyters integritet och skulle kunna störa vissa analysteknologier[40]. Eftersom det finns så många olika anordningar för provinsamling, kan Ortho-Clinical Diagnostics inte avge något definitivt utlåtande om dess produkters prestanda tillsammans med dessa anordningar. Det rekommenderas att varje användare tillser att den anordning som valts används i enlighet med tillverkarens anvisningar och att den är kompatibel med denna *Vitros*-analys.

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Kvalitetskontroll och noteringar om proceduren

- Hantera reagenspack försiktigt, undvik följande: låta kondens bildas på pack; få reagensen att skumma; agitera pack.
- Kalibrering är lot-specifik; reagenspack och kalibratorer är förenade via lot-numret. Reagenspack inom en lot kan använda samma kalibrering, som måste utföras med en kalibrator med samma lot-nummer.
- Blanda prov, kalibratorer och kontroller noga genom inversion och låt dem komma upp i 15-30°C före användning.
- Hantera prov, kalibratorer och kontroller i proppade behållare för att undvika kontamination och avdunstning. Undvik avdunstning genom att begränsa hur länge prov, kalibratorer och kontroller befinner sig på *Vitros*-systemet. Se användarhandboken till *Vitros*-systemet för mer information. Återför till 2-8 °C snarast möjligt efter användning, eller ladda bara tillräckligt för en enda användning. Kalibratorn kan fördelas i alikvoter i alternativa behållare, vilka kan streckkodas med bifogade etiketter.
- Anti-HCV-kalibratorn duplikatanalyseras automatiskt.
- Kontrollera förrådet regelbundet för att underlätta hanteringen av reagens och se till att det finns tillräckligt med Vitros Signalreagens, Vitros tvättreagens och kalibrerade reagens-loter för det planerade arbetet. Vid utförandet av analyspaneler på ett enstaka prov, kontrollera att provvolymen är tillräcklig för de beställda analyserna.
- Enligt god laboratoriesed måste kontroller analyseras för att verifiera analysens prestanda. Det finns 2 *Vitros* anti-HCV-kontroller (anti-HCV negativ och anti-HCV positiv). Det rekommenderas att kontroller analyseras efter kalibrering, och därefter minst en gång per 24 timmar och efter utförandet av vissa serviceprocedurer (Se användarhandboken till *Vitros*-systemet). Om kvalitetskontrollprocedurer på ditt laboratorium erfordrar tätare användning av kontroller, följ dessa procedurer. För mer detaljerad information, se användarhandboken till *Vitros*-systemet.
- Standardanalysnamnet som visas på patientrapporter är Anti-HCV. Det förkortade standardnamnet som visas i analysvalmenyer och laboratorierapporter är aHCV. Dessa standardinställningar kan omkonfigureras vid behov i menyn Alternativ & Konfiguration - Konfig. analyser.

Procedur

För *Vitros* anti-HCV-analys behövs 20 µL prov, kalibrator eller kontroll för en enstaka bestämning. Då är inte dödvolymen för den valda provbehållaren medräknad.

Vitros anti-HCV-analys måste kalibreras varje gång en ny reagens-lot tas i bruk, och därefter med intervall på 28 dagar. *Vitros* anti-HCV-analys kan även behöva kalibreras efter utförandet av vissa serviceprocedurer eller om kvalitetskontrollresultat konsekvent ligger utanför godtagbart område.

För detaljanvisningar om användningen av systemet, se användarhandboken till *Vitros* immunodiagnostiksystem, kapitel 4-7. I sammanfattning:

1. Skanna protokollkortet för att överföra ett nytt analysprotokoll till systemet. Därefter visas analysknappen i menyn Prov programmering. Skanna lot-kalibreringskortet för varje ny reagens-lot för att lägga in lot-specifik information om kalibrering och utgångsdatum.
2. Öppna foliepåsen och ta ut reagenspack. Ladda pack vid auto-load-stationen, eller använd knappen Ladda ur/Ladda på skärmen Reagenshantering – Visa enl. analys. **Obs!** Använd inte en produkt som är skadad eller ofullständigt förseglad.
3. Ladda prov i provrack, och använd adaptrar vid behov (prov kan streckkodas om man så önskar). Placera en engångsspets i anslutning till varje prov och ladda rack i systemet. Utför beställning i menyn Prov programmering. Starta provuppmätningen, alla provanalyseringssteg utförs automatiskt.
4. Analysera kalibratorn på samma sätt som prov (ladda tillräckligt för den automatiska analyseringen i duplikat). Kalibrering behöver inte programmeras om streckkodsetiketter används, kalibrering startas automatiskt.

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Resultat

Resultat beräknas som en normaliserad signal, i relation till ett cut-off-värde. Under kalibreringsprocessen används en lot-specifik parameter, som är kodad på lot-kalibreringskortet, för att bestämma ett giltigt lagrat cut-off-värde för systemet. För ytterligare information om kalibrering, se bruksanvisningen som medföljer anti-HCV-kalibratorn. Resultaten beräknas automatiskt av *Vitros*-systemet.

$$\text{Resultat} = \frac{\text{Signal för analyserat prov}}{\text{Cut-off-värde}}$$

Resultatet ≥1,00 indikerar ett reaktivt prov och eventuell förekomst av anti-HCV.
Resultatet <0,90 indikerar ett icke-reaktivt prov, negativt beträffande anti-HCV.
Resultatet ≥0,90 och <1,00 indikerar ett borderline-prov.

Kvalitetskontroll

- Kalibreringsresultat bedöms mot två kvalitetsparametrar som beskrivs i Visa kal parametrar, som kan nås via skärmen Alternativ & Konfiguration –Granska/ Användarkalibrering. Ett misslyckande att uppnå endera av de definierade kvalitetsparameterområdena kodas i kalibreringsrapporten. För åtgärder som skall vidtas efter en misslyckad kalibrering, se användarhandboken, kapitel 8, Granska resultat.
- Patientresultat flaggmarkeras som 'Negative', 'Borderline' eller 'Reactive'. Kontrollvärden flaggas vid ≥2 standardavvikelser från definierat baslinje-medelvärde.
- Kalibreringens kvalitet kan inte beskrivas helt med en enda parameter. Kalibreringsrapporten skall användas tillsammans med kontrollvärden för att avgöra kalibreringens validitet.
- Om kontrollresultat hamnar utanför godtagbart område, undersök orsaken innan patientresultat rapporteras.

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Procedurens begränsningar

- Resultat från detta eller något annat diagnostiskt kit skall endast användas och tolkas inom ramen för den totala kliniska bilden. Ett negativt testresultat utesluter inte möjligheten till exponering för eller infektion med HCV. HCV-antikroppar kan vara omöjliga att detektera i vissa infektionsstadier och vid vissa kliniska tillstånd[7].
- Heterofila antikroppar i serum eller plasmaprov kan interferera i immunoanalyser[8]. Dessa antikroppar kan finnas i blodprov från individer som regelbundet exponeras för djur eller som behandlats med animaliska serumprodukter. Resultat som är oförenliga med kliniska observationer indikerar behovet av ytterligare analyser.
- Prov som innehåller triolein (<33,9 mmol/L), hemoglobin (<5 g/L) eller bilirubin (<0,342 mmol/L) stör inte den kliniska tolkningen av dessa resultat. Använd inte grumliga prov.

Tolkning av resultat

Ett prov som befinns vara borderline eller reaktivt måste testas igen i duplikat för att verifiera dess status. Före ny analys skall provet centrifugeras så att det är fritt från celler, cellfragment och fibrin. Om resultaten vid upprepad analys är <0,90 för båda replikaten, ska provet betraktas som negativt. Om ett av de duplicerade nya analysresultaten är ≥0,90, ska provet analyseras med kompletterande analysmetoder för att bekräfta resultatet. Ett upprepat reaktivt prov som bekräftats med extra analyser måste betraktas som positivt beträffande anti-HCV. I fall av upprepade borderline-resultat rekommenderas analys av uppföljningsprov.

Prestandakarakteristik

Kalibrering och kalibreringens spårbarhet
Kalibreringen av *Vitros* anti-HCV-analys kan spåras till en intern referenskalibrator som har tilldelats ett värde för att optimera kliniska sensitivitets- och specificitetsprestanda.

Precision
Precision utvärderades i enlighet med protokollet från National Committee for Clinical Laboratory Standards EP5-T2[9]. Två replikat av vardera 4 panelprov analyserades vid ett tillfälle per dag i minst 20 dagar. Experimentet utfördes med användning av 2 reagensloter i olika system. De data som presenteras är representativa för produktens prestanda.

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Tabell 1: Precision

Representativt resultat	Inom körning		Inom kalibrering		Inom laboratorium	
	SD*	CV(%)*	SD*	CV(%)*	SD*	CV(%)*
0,14	0,00336	2,4	0,00835	4,9	0,00945	6,9
2,04	0,044	2,1	0,132	6,4	0,134	6,6
5,74	0,132	2,4	0,339	5,8	0,354	6,2
14,9	0,187	1,2	0,454	3,1	0,576	3,9

* Effektivvärde (root mean square, RMS)

Inom körning: Duplikatprecision fördelad över alla körningar.
Inom kalibrering: Total precision, med viktade komponenter för precision inom serie och mellan dagar.
Inom laboratorium: Ett mått på effekten av omkalibrering på total precision, beräknat inom reagens-lot, med användning av data från 4 kalibreringar.

Sensitivitet
435 patientprov som tidigare bestämts som positiva med en HCV immunoblot-assay analyserades i *Vitros* anti-HCV-analys. Sensitiviteten för denna provpopulation i *Vitros* anti-HCV-analys beräknades till 100 % (435/435).

Dessutom analyserades 29 kommersiellt tillgängliga serokonversionspaneler. *Vitros* anti-HCV-analys visade likvärdig eller högre serokonversionssensitivitet beträffande 29/29 paneler, jämfört med de publicerade resultaten från en annan kommersiellt tillgänglig analys.

Specificitet
Prov från 5 374 förmodat friska blodgivare och 393 kliniska prov analyserades i *Vitros* anti-HCV-analys och i en annan kommersiellt tillgänglig analys.

Prov	Antal analysprov	Initialt reaktiva	Upprepat reaktiva	Bekräftat positiva
Givare	5 374	14	13	0
Kliniskt	393	1	1	0

SV

Specificiteten för *Vitros* anti-HCV-analys för givarproven beräknades till 99,76 % (5 361/5 374) baserat på upprepad reaktivitet. Specificiteten för *Vitros* anti-HCV-analys för givarproven beräknades till 99,75 % (392/393) baserat på upprepad reaktivitet.

Ytterligare 161 prov från följande potentiellt korsreagerande subgrupper analyserades med *Vitros* anti-HCV-analys: CMV-positiva, EBV-positiva, HIV-positiva, patienter med icke-viral leversjukdom, andra virala leversjukdomar (t.ex HBV, HAV), SLE, reumafaktorpositiva, nyvaccinerade (t.ex. mot influensa), jästreaktiva prov. Av dessa kategorier var det ingen som resulterade i falska reaktiva resultat i *Vitros* anti-HCV-analys.

ADM-arbetsgrupp: Sammanfattning av utvärdering

Vitros anti-HCV-analys utvärderades av ADM-arbetsgruppen. Man analyserade 450 prov från 246 patienter som var infekterade med olika genotyper av hepatit C-virus och 50 prov från HCV SFTS-panelen, inklusive 27 RNA-reaktiva prov. *Vitros* anti-HCV-analys visade god sensitivitet beträffande detektionen av tidig serokonversion och beträffande screeningen av andra kategorier av reaktiva prov, inklusive de som visar upp en isolerad reaktivitet och de som kommer från kroniska bärare.

En population av 2 018 blodgivarprov analyserades med *Vitros* anti-HCV-analys. Åtta prov gav upprepningsbara reaktiva resultat, av vilka 1 bekräftades som reaktivt och 6 befanns vara negativa med andra analyser. Resultatet för det återstående provet kunde inte tolkas (obestämbart NS3, PCR-negativt). Specificiteten för *Vitros* anti-HCV-analys var cirka 99,70 %.

Licensmeddelande

HCV-rekombinanta antigener som används i *Vitros* anti-HCV-analys bereds under amerikansk licens av Chiron Corporation enligt ett gemensamt tillverkningsavtal.

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Vitros Immunodiagnostic Products
Πακέτο αντιδραστηρίου αντι-HCV

Ενδεικνυόμενη Χρήση

Για την *in vitro* ποιοτική ανίχνευση αντισωμάτων έναντι του ιού της ηπατίτιδας C (αντι-HCV) σε ανθρώπινο ορό και πλάσμα (EDTA, ηπαρίνη ή κιτρικά).

Περίληψη και επεξήγηση της εξέτασης

Είναι πλέον γνωστό ότι ο ιός της ηπατίτιδας C (HCV) αποτελεί τον αιτιολογικό παράγοντα για τις περισσότερες, αν όχι όλες, μεταδιδόμενες μέσω του αίματος μη A, μη B ηπατίτιδες (NANBH). Μελέτες σε όλο τον κόσμο αποδεικνύουν ότι ο ιός HCV μεταδίδεται μέσω μολυσμένου αίματος και παραγώγων αίματος, μέσω των μεταγγίσεων αίματος ή μέσω στενών προσωπικών επαφών. Η παρουσία του αντι-HCV υποδηλώνει ότι ένα άτομο ενδέχεται να έχει μολυνθεί από τον HCV και να είναι ικανό να μεταδώσει τη λοίμωξη HCV[1].

Στον προσδιορισμό αντι-HCV *Vitros* χρησιμοποιούνται τρία ανασυνδυασμένα αντιγόνα, κωδικοποιούμενα από τον ιό της ηπατίτιδας C. Τα τρία ανασυνδυασμένα αντιγόνα είναι τα c22-3, c200 και NS-5. Η ανασυνδυασμένη πρωτεΐνη c22-3 κωδικοποιείται από τη θεωρούμενη πυρηνική περιοχή του γονιδιώματος του HCV. Η ανασυνδυασμένη πρωτεΐνη c200 του HCV κωδικοποιείται από τις NS3 και NS4 θεωρούμενες περιοχές του γονιδιώματος του HCV. Η πρωτεΐνη c200 εμπεριέχει την αλληλουχία πρωτεΐνών c33c η οποία είναι γενετικά συνδεδεμένη με την αλληλουχία πρωτεΐνών c100-3. Μελέτες έχουν δείξει ότι τα αντισώματα τα οποία αναπτύσσονται μετά τη μόλυνση από τον HCV είναι συχνά αντιδρώντα με τις πρωτεΐνες c22-3 ή/και c33c[2]. Η ανασυνδυασμένη πρωτεΐνη NS5 του HCV κωδικοποιείται από την NS5 θεωρούμενη περιοχή του γονιδιώματος του HCV. Ένας σημαντικός αριθμός ατόμων που έχουν μολυνθεί από τον HCV αναπτύσσουν αντισώματα έναντι της NS5[3].

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Ο οικοδεσπότης οργανισμός και για τα τρία ανασυνδυασμένα αντιγόνα του HCV είναι ο οργανισμός *S. cerevisiae* (ζυμομύκητας).

Αρχές της διαδικασίας

Ο προσδιορισμός αντι-HCV *Vitros* πραγματοποιείται με τη χρήση του πακέτου αντιδραστηρίου αντι-HCV *Vitros* και του βαθμονομητή αντι-HCV των ανοσοδιαγνωστικών προϊόντων *Vitros* στο ανοσοδιαγνωστικό σύστημα *Vitros*.

Χρησιμοποιείται μια ανοσομετρική τεχνική η οποία περιλαμβάνει αντίδραση σε δύο στάδια. Στο πρώτο στάδιο, το αντίσωμα έναντι του HCV που βρίσκεται στο δείγμα δεσμεύεται με τα ανασυνδυασμένα αντιγόνα του HCV στα βυθίσματα . Το αδέσμευτο δείγμα αφαιρείται μέσω έκπλυσης. Στο δεύτερο στάδιο, το σημασμένο με ραφανιδική υπεροξειδάση (HRP) συζυγές αντίσωμα (μονοκλωνικό αντίσωμα IgG ποντικού) δεσμεύεται σε οποιαδήποτε ανθρώπινη IgG η οποία έχει συλληφθεί πάνω στο φρεάτιο κατά το πρώτο στάδιο. Το αδέσμευτο συζυγές αφαιρείται μέσω έκπλυσης.

Η μέτρηση του σημασμένου με HRP συζυγούς αντισώματος γίνεται μέσω μιας αντίδρασης φωταύγειας[4]. Ένα αντιδραστήριο που περιέχει φωτογενή υποστρώματα (ένα φωτεινό παράγωγο και ένα άλας υπεροξέος) και ένας παράγοντας μεταφοράς ηλεκτρονίων, προστίθενται στα βυθίσματα. Η HRP στο δεσμευμένο συζυγές καταλύει την οξείδωση του φωτεινού παραγώγου, παράγοντας φως. Ο παράγοντας μεταφοράς ηλεκτρονίων (μια υποκατεστημένη άχρωμη κρυσταλλική σκόνη) αυξάνει το επίπεδο φωτός που δημιουργείται και παρατείνει την εκπομπή του. Τα φωτεινά σήματα αναγνωρίζονται από το Σύστημα *Vitros*. Η ποσότητα του σημασμένου με HRP δεσμευμένου συζυγούς είναι ευθέως ανάλογη προς τη συγκέντρωση αντι-HCV στο δείγμα.

Προειδοποιήσεις και προφυλάξεις

Για *In Vitro* διαγνωστική χρήση μόνο

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Προειδοποίηση – Δυνητικώς μολυσματικό υλικό

- Τα προϊόντα πρέπει να θεωρούνται ικανά να μεταδώσουν μόλυνση και ο χειρισμός τους πρέπει να γίνεται ανάλογα.
- Ο χειρισμός των δειγμάτων και των εξαρτημάτων του προσδιορισμού, η χρήση και η αποθήκευσή τους, καθώς και η απόρριψη στερεών και υγρών αποβλήτων θα πρέπει να γίνεται σύμφωνα με τις διαδικασίες που καθορίζονται από την ανάλογη εθνική οδηγία ή κανονισμό σχετικά με την ασφάλεια έναντι μολυσματικών υλικών (π.χ. οδηγία NCCLS M29[53]).

Ο βαθμονομητής αντι-HCV *Vitros* περιέχει:
Πλάσμα θετικό για αντι-HCV που έχει ληφθεί από δότες στους οποίους πραγματοποιήθηκε μεμονωμένος έλεγχος με τη χρήση εγκεκριμένων μεθόδων (ενζυμικοί ανοσοπροσδιορισμοί) και οι οποίοι βρέθηκε ότι είναι αρνητικοί για το επιφανειακό αντιγόνο της ηπατίτιδας Β και για αντισώματα έναντι του ιού της ανοσοανεπάρκειας του ανθρώπου (HIV 1+2). Το θετικό για αντι-HCV πλάσμα έχει υποστεί επεξεργασία με σκοπό να μειωθεί ο τίτλος τού δυνητικώς μολυσματικού ιού. Ωστόσο, επειδή καμία μέθοδος δοκιμασίας δεν μπορεί να αποκλείσει τον κίνδυνο μόλυνσης, να χειρίζεστε το προϊόν ως ικανό να μεταδώσει μόλυνση.

Πλάσμα αρνητικό για αντι-HCV που έχει ληφθεί από δότες στους οποίους πραγματοποιήθηκε μεμονωμένος έλεγχος με τη χρήση εγκεκριμένων μεθόδων (ενζυμικοί ανοσοπροσδιορισμοί) και οι οποίοι βρέθηκε ότι είναι αρνητικοί για το επιφανειακό αντιγόνο της ηπατίτιδας Β και για αντισώματα έναντι του ιού της ηπατίτιδας C και του ιού της ανοσοανεπάρκειας του ανθρώπου (HIV 1+2).

Θα πρέπει να δίνεται προσοχή κατά τον χειρισμό υλικού ανθρώπινης προέλευσης. Έλατα δείγματα θα πρέπει να θεωρούνται δυνητικώς μολυσματικά. Καμία εργαστηριακή μέθοδος δεν μπορεί να προσφέρει απόλυτη εγγύηση ότι οι ιοί HBV, HCV, HIV 1+2 ή άλλοι μολυσματικοί παράγοντες είναι απόντες.

Προειδοποίηση – Περιέχει ProClin 300 και 2-Χλωροακεταμίδιο
Το αντιδραστήριο συζυγούς περιέχει ProClin 300 και το αντιδραστήριο προσδιορισμού περιέχει 2-Χλωροακεταμίδιο. R43: Μπορεί να προκαλέσει ευαισθητοποίηση σε επαφή με το δέρμα. R52/53: Επιβλαβές για τους υδρόβιους οργανισμούς, μπορεί να προκαλέσει μακροχρόνιες δυσμενείς επιπτώσεις στο υδάτινο περιβάλλον.
S24: Αποφεύγετε την επαφή με το δέρμα. S37: Να φοράτε κατάλληλα γάντια.

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Παρεχόμενα υλικά

1 πακέτο αντιδραστηρίου περιέχει:

- 100 επικαλυμμένα φρεάτια (Ανασυνδυασμένα αντιγόνα ιού ηπατίτιδας C προερχόμενα από ζύμη. Επικάλυψη 0,41 μg/φρεάτιο).
- 18,2 mL αντιδραστηρίου προσδιορισμού - (ρυθμιστικό διάλυμα με αντιμικροβιακό παράγοντα).
- 20,6 mL αντιδραστηρίου συζυγούς - (σημασμένο με ραφανιδική υπεροξειδάση [HRP] μονοκλωνικό αντίσωμα IgG ποντικού, 1,04 ng/φρεάτιο) σε ορό εμβρύου μόσχου με ρυθμιστικό διάλυμα και αντιμικροβιακό παράγοντα.

Σημείωση: Περιέχει λευκωματίνη βοείου ορού και ορό εμβρύου μόσχου.

Απαιτούμενα υλικά που δεν παρέχονται

Το ανοσοδιαγνωστικό σύστημα *Vitras* και τα ακόλουθα ανοσοδιαγνωστικά προϊόντα *Vitras*: Βαθμονομητής αντι-HCV (περιλαμβανομένης της κάρτας βαθμονόμησης παρτίδας και της κάρτας πρατοκόλλου), αντιδραστήριο σήματος, γενικό αντιδραστήριο έκπλυσης, κουτί φύλαξης πακέτων αντιδραστηρίου (προαιρετικό) με αποξηραντική ουσία.

Προετοιμασία και αποθήκευση αντιδραστηρίου

Το πακέτο αντιδραστηρίου παρέχεται έτοιμο προς χρήση. Αποθηκεύστε με κλειστή συσκευασία σε θερμοκρασία 2-8°C, μην καταψύχετε. Χρησιμοποιήστε τα πακέτα αντιδραστηρίου που έχουν ανοιχτεί, μέσα σε διάστημα 8 εβδομάδων από την πρώτη φόρτωση στο σύστημα. Μη χρησιμοποιείτε μετά την ημερομηνία λήξης. Αποθηκεύστε πάνω στο σύστημα τα πακέτα αντιδραστηρίου που έχουν ανοιχτεί ή σε θερμοκρασία 2-8°C μέσα σε ένα σφραγισμένο κουτί αποθήκευσης πακέτων αντιδραστηρίου το οποίο να περιέχει κάποια αποξηραντική ουσία.

Ο βαθμονομητής αντι-HCV παρέχεται έτοιμος προς χρήση. Αποθηκεύστε με κλειστή συσκευασία σε θερμοκρασία 2-8°C. Μη χρησιμοποιείτε μετά την ημερομηνία λήξης. Μετά το άνοιγμα της συσκευασίας, φυλάξτε το προϊόν για διάστημα έως 13 εβδομάδων σε θερμοκρασία 2-8°C ή για 13 εβδομάδες στους -20°C (με όχι παραπάνω από 1 κύκλο ψύξης-απόψυξης).

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Προετοιμασία ασθενούς

Δεν απαιτείται ειδική προετοιμασία του ασθενούς.

Συλλογή δείγματος, προετοιμασία και αποθήκευση

Μπορούν να χρησιμοποιηθούν δείγματα ορού ή πλάσματος (EDTA, ηπαρίνη ή κιτρικά). Τα αποτελέσματα από δείγματα πλάσματος με κιτρικά θα είναι αναλογικά χαμηλότερα λόγω της αραίωσης από το υγρό αντιπηκτικό. Συλλέξτε δείγματα αίματος χρησιμοποιώντας καθιερωμένες διαδικασίες. Τα δείγματα θα πρέπει να διαχωρίζονται εντελώς από το κυτταρικό υλικό. Αν δεν πραγματοποιηθεί αυτός ο διαχωρισμός, ενδέχεται να προκύψουν ψευδώς αυξημένα αποτελέσματα. Τα δείγματα πλάσματος με ηπαρίνη μπορούν να αποθηκευτούν για διάστημα έως και 7 ημερών σε θερμοκρασία 2-8°C ή έως και 4 εβδομάδων στους -20°C.

Έχει αναφερθεί κάποιες φορές ότι οι συσκευές συλλογής δειγμάτων είναι επιβλαβείς όσον αφορά την ακεραιότητα ορισμένων αναλυόμενων ουσιών και μπορεί να παρέμβουν σε ορισμένες μεθόδους[6]. Λόγω της ποικιλίας των συσκευών συλλογής δειγμάτων που υπάρχουν διαθέσιμες, η Ortho-Clinical Diagnostics δεν έχει τη δυνατότητα να παράσχει μια οριστική δήλωση σχετικά με την απόδοση των προϊόντων της με αυτές τις συσκευές. Συνιστάται κάθε χρήστης να διασφαλίζει ότι η επιλεγμένη συσκευή χρησιμοποιείται σύμφωνα με τις οδηγίες του κατασκευαστή και είναι συμβατή με τον προσδιορισμό *Vitros*.

Ποιοτικός έλεγχος και σημειώσεις για τη διαδικασία

- Να χειρίζεστε το πακέτο αντιδραστηρίου με προσοχή, αποφεύγοντας τα ακόλουθα: Την ανακίνηση του πακέτου και το σχηματισμό συμπύκνωσης στο πακέτο η οποία θα προκαλέσει τη δημιουργία αφρού στα αντιδραστήρια.
- Η βαθμονόμηση είναι ειδική για κάθε παρτίδα. Τα πακέτα αντιδραστηρίου και οι βαθμονομητές συνδέονται με τον αριθμό παρτίδας. Τα πακέτα αντιδραστηρίου από την ίδια παρτίδα μπορούν να χρησιμοποιήσουν την ίδια βαθμονόμηση η οποία πρέπει να πραγματοποιηθεί με τη χρήση ενός βαθμονομητή από τον ίδιο αριθμό παρτίδας.
- Αναμείξτε πλήρως τα δείγματα, τους βαθμονομητές και τα υλικά ελέγχου με ανάδευση και αφήστε να αποκτήσουν θερμοκρασία 15-30°C πριν από τη χρήση.

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- Ο χειρισμός των δειγμάτων, των βαθμονομητών και των ορών ελέγχου πρέπει να γίνεται σε δοχεία με τάπα προκειμένου να αποφεύγεται η μόλυνση και η εξάτμιση. Για να αποφευχθεί η εξάτμιση, περιορίστε το διάστημα κατά το οποίο τα δείγματα, οι βαθμονομητές και οι έλεγχοι βρίσκονται πάνω στο σύστημα *Vitros*. Για επιπλέον πληροφορίες, ανατρέξτε στον Οδηγό Χειριστή του Συστήματος *Vitros*. Αφήστε τους βαθμονομητές να αποκτήσουν θερμοκρασία 2-8 °C το συντομότερο δυνατόν μετά τη χρήση ή φορτώστε επαρκές διάλυμα για μία χρήση μόνο. Ο βαθμονομητής μπορεί να υποδιαιρεθεί σε εναλλακτικά δοχεία στα οποία είναι δυνατόν να τοποθετηθούν οι ετικέτες ραβδοκώδικα που παρέχονται.
- Η επεξεργασία του βαθμονομητή αντι-HCV γίνεται αυτομάτως εις διπλούν.
- Κάνετε τακτικά έλεγχο απογραφής προκειμένου να βοηθήσετε το χειρισμό των αντιδραστηρίων και να διασφαλίσετε ότι υπάρχουν διαθέσιμες παρτίδες Αντιδραστηρίου Σήματος *Vitros*, Γενικού Αντιδραστηρίου Πλύσης *Vitros* και βαθμονομημένων αντιδραστηρίων για την εργασία που έχει προγραμματιστεί. ταν πραγματοποιείτε μια σειρά προσδιορισμών σε ένα μόνο δείγμα, βεβαιωθείτε ότι ο όγκος του δείγματος είναι επαρκής για τους προσδιορισμούς που έχουν ζητηθεί.
- Η ορθή εργαστηριακή πρακτική απαιτεί να πραγματοποιούνται έλεγχοι για να επαληθεύεται η απόδοση του προσδιορισμού. Υπάρχουν δύο υλικά ελέγχου (μάρτυρες) αντι-HCV *Vitros* (ένα αρνητικό για αντι-HCV και ένα θετικό για αντι-HCV). Συνιστάται οι έλεγχοι να εκτελούνται κατά την πραγματοποίηση μιας βαθμονόμησης, και ακολούθως τουλάχιστον μια φορά κάθε 24 ώρες και μετά την πραγματοποίηση καθορισμένων διαδικασιών επισκευής (Ανατρέξτε στον Οδηγό Χειριστή του Συστήματος *Vitros*). Αν οι διαδικασίες ελέγχου ποιότητας που ισχύουν στο εργαστήριό σας απαιτούν να γίνονται συχνότεροι έλεγχοι, ακολουθήστε αυτές τις διαδικασίες. Για πιο λεπτομερείς πληροφορίες, ανατρέξτε στον Οδηγό Χειριστή του Συστήματος *Vitros*.
- Το προεπιλεγμένο όνομα προσδιορισμού που θα εμφανιστεί στα αποτελέσματα του ασθενούς είναι Anti-HCV. Το προεπιλεγμένο σύντομο όνομα που θα εμφανιστεί στα μενού επιλογής του προσδιορισμού και στις εκθέσεις του εργαστηρίου είναι aHCV. Αυτές οι προεπιλογές μπορούν να αναδιαμορφωθούν, αν απαιτείται, στην οθόνη "Επιλογές και Διαμόρφωση – Διαμόρφωση αναλυόμενης ουσίας".

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Διαδικασία

Ο προσδιορισμός αντι-HCV *Vitros* απαιτεί 20 µL δείγματος, βαθμονομητή ή υλικού ελέγχου για προσδιορισμό σε ένα μόνο αντίγραφο. Σε αυτό δεν λαμβάνεται υπόψη ο ελάχιστος όγκος πλήρωσης του επιλεγμένου δοχείου δειγμάτων.

Ο προσδιορισμός αντι-HCV *Vitros* πρέπει να βαθμονομείται κάθε φορά που χρησιμοποιείται μια νέα παρτίδα αντιδραστηρίου και ακολούθως κατά διαστήματα 28 ημερών. Ο προσδιορισμός αντι-HCV *Vitros* ενδέχεται να χρειαστεί να βαθμονομηθεί μετά την πραγματοποίηση ορισμένων εργασιών συντήρησης ή σε περίπτωση που τα αποτελέσματα του ποιοτικού ελέγχου βρίσκονται σταθερά εκτός των αποδεκτών ορίων σας.

Για λεπτομερείς οδηγίες σχετικά με τη λειτουργία του Συστήματος, ανατρέξτε στα κεφάλαια 4-7 του Οδηγού Χειριστή του Συστήματος *Vitros*. Εν συντομία:

1. Σαρώστε την κάρτα πρωτοκόλλου για να φορτώσετε έναν νέο πρωτόκολλο προσδιορισμού στο Σύστημα. Στη συνέχεια, το πλήκτρο προσδιορισμού εμφανίζεται στην οθόνη "Ενδεικτικός Προγραμματισμός". Σαρώστε την κάρτα διαβάθμισης παρτίδας για κάθε νέα παρτίδα αντιδραστηρίου για να εισαγάγετε τις ειδικές για την παρτίδα πληροφορίες βαθμονόμησης και λήξης.
2. Ανοίξτε το αλουμινένιο σακουλάκι και αφαιρέστε το πακέτο αντιδραστηρίου. Φορτώστε το πακέτο στο σταθμό αυτοφόρτωσης ή χρησιμοποιήστε το πλήκτρο **Unload/Load** στην οθόνη "Διαχείριση Αντιδραστηρίων – Εμφάνιση κατά Αντιδραστήριο". **Σημείωση:** Μη χρησιμοποιείτε κατεστραμμένα ή ατελώς σφραγισμένα προϊόντα.
3. Φορτώστε τα δείγματα στους δίσκους γενικού δείγματος χρησιμοποιώντας κάποιον προσαρμογέα όποτε απαιτείται (τα δείγματα μπορούν να φέρουν ραβδοκώδικα, αν το επιθυμείτε). Τοποθετήστε ένα αναλώσιμο ρύγχος δίπλα σε κάθε δείγμα και φορτώστε τους δίσκους στο Σύστημα. Καθορίστε τα προγράμματα δείγματος χρησιμοποιώντας την οθόνη "Ενδεικτικός Προγραμματισμός". Ξεκινήστε τη λειτουργία ανάλυσης των δειγμάτων. Όλα τα βήματα της επεξεργασίας των δειγμάτων θα πραγματοποιηθούν αυτομάτως.
4. Επεξεργαστείτε το βαθμονομητή με τον ίδιο τρόπο που επεξεργαστήκατε τα δείγματα (φορτώνοντας επαρκή ποσότητα για τον αυτόματο προσδιορισμό εις διπλούν). Δεν χρειάζεται να προγραμματιστεί η βαθμονόμηση αν χρησιμοποιούνται ετικέτες ραβδοκώδικα. Η βαθμονόμηση θα ξεκινήσει αυτομάτως.

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Αποτελέσματα

Τα αποτελέσματα υπολογίζονται ως ένα κανονικοποιημένο σήμα, σε σχέση με μια τιμή αποκοπής. Κατά τη διάρκεια της διαδικασίας της βαθμονόμησης χρησιμοποιείται μια ειδική για την παρτίδα παράμετρος που υπάρχει κωδικοποιημένη στην κάρτα διαβάθμισης παρτίδας, προκειμένου να προσδιορίσει μια έγκυρη, αποθηκευμένη τιμή αποκοπής για το Σύστημα. Για περισσότερες λεπτομέρειες σχετικά με τη βαθμονόμηση, ανατρέξτε στις οδηγίες χρήσης που παρέχονται με το βαθμονομητή αντι-HCV. Τα αποτελέσματα υπολογίζονται αυτομάτως από το Σύστημα *Vitros*.

$$\text{Αποτέλεσμα} = \frac{\text{Σήμα για το δείγμα εξέτασης}}{\text{Τιμή αποκοπής}}$$

Ένα αποτέλεσμα ≥1,00 υποδεικνύει ένα αντιδρών δείγμα και την πιθανή παρουσία του αντι-HCV.
Ένα αποτέλεσμα <0,90 υποδεικνύει έναμη αντιδρών δείγμα, αρνητικό για το αντι-HCV.
Ένα αποτέλεσμα ≥0,90 και <1,00 υποδεικνύει ένα οριακό δείγμα.

Έλεγχος ποιότητας

- Τα αποτελέσματα βαθμονόμησης αξιολογούνται έναντι δύο παραμέτρων ποιότητας, όπως παρουσιάζεται αναλυτικά στο View Cal Parameters (Εμφάνιση Παραμέτρων Βαθμονόμησης), μέσω της οθόνης Options & Configuration - Review/User Calibrations (Επιλογές και Διαμόρφωση--Ανασκόπηση/Βαθμονόμηση χρήστη). Αν δεν καλυφθεί το μετρούμενο εύρος τιμών για κάποια από τις παραμέτρους ποιότητας, αυτό θα σημειωθεί στην αναφορά της βαθμονόμησης. Για ενέργειες που πρέπει να πραγματοποιηθούν έπειτα από μια αποτυχημένη βαθμονόμηση, ανατρέξτε στο Κεφάλαιο 8 (Ανασκόπηση Αποτελεσμάτων) του Οδηγού Χειριστή.
- Οι τιμές του δείγματος ασθενούς θα σημειωθούν ως "Negative" (Αρνητικές), "Borderline" (Οριακές) ή "Reactive" (Αντιδράσεις). Οι τιμές ελέγχου θα σημειωθούν όταν είναι ≥2 SDs (τυπική απόκλιση) από την καθορισμένη μέση γραμμή βάσης.
- Η ποιότητα της βαθμονόμησης δεν είναι δυνατόν να περιγραφεί πλήρως μέσω μίας μόνον παραμέτρου. Η αναφορά της βαθμονόμησης θα πρέπει να χρησιμοποιείται σε συνδυασμό με τις τιμές ελέγχου προκειμένου να προσδιορίζεται η εγκυρότητα της βαθμονόμησης.

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- Αν τα αποτελέσματα ελέγχου βρεθούν εκτός των αποδεκτών ορίων σας, διερευνήστε την αιτία πριν αποφασίσετε αν θα πρέπει να αναφέρετε τα αποτελέσματα του ασθενούς.

Περιορισμοί της διαδικασίας

- Τα αποτελέσματα από αυτό ή οποιοδήποτε άλλο διαγνωστικό κιτ θα πρέπει να χρησιμοποιούνται και να ερμηνεύονται μόνο στο πλαίσιο της συνολικής κλινικής εικόνας του ασθενούς. Ένα αρνητικό αποτέλεσμα δοκιμασίας δεν αποκλείει την πιθανότητα έκθεσης στον ιό της ηπατίτιδας C ή μόλυνσης από αυτόν. Τα αντισώματα έναντι του HCV ενδέχεται να μην είναι ανιχνεύσιμα σε κάποια στάδια της λοίμωξης και σε ορισμένες κλινικές καταστάσεις[7].
- Τα ετερόφιλα αντισώματα σε δείγματα ορού ή πλάσματος ενδέχεται να προκαλέσουν παρέμβαση στους ανοσοπροσδιορισμούς[9]. Τα αντισώματα αυτά ενδέχεται να είναι παρόντα σε δείγματα αίματος από άτομα που έρχονται σε επαφή με ζώα ή που έχουν ακολουθήσει θεραπευτική αγωγή με προϊόντα από ορό ζώων. Τα αποτελέσματα τα οποία είναι ασύμφωνα προς τις κλινικές παρατηρήσεις υποδεικνύουν την ανάγκη για περαιτέρω εξέταση.
- Τα δείγματα που περιέχουν τριελαΐνη (<33,9 mmol/L), αιμοσφαιρίνη (<5 g/L) ή χολερυθρίνη (<0,342 mmol/L) δεν παρεμβαίνουν στην κλινική ερμηνεία των αποτελεσμάτων αντών. Μη χρησιμοποιείτε θολά δείγματα.

Ερμηνεία των αποτελεσμάτων

Ένα δείγμα που θα βρεθεί να είναι οριακό ή αντιδρών, θα πρέπει να επανεξεταστεί εις διπλούν προκειμένου να επαληθευτεί η κατάστασή του. Πριν γίνει η επανεξέταση, το δείγμα θα πρέπει να υποστεί φυγοκέντρηση για να εξασφαλιστεί η απαλλαγή του από κύτταρα, κυτταρικά υπολείμματα ή ινώδες. Αν τα αποτελέσματα στην επαναληπτική εξέταση είναι <0,90 και για τα δύο αντίγραφα, το δείγμα θα πρέπει να θεωρηθεί αρνητικό. Αν το αποτέλεσμα της επαναληπτικής δοκιμασίας οποιουδήποτε από τα αντίγραφα είναι ≥0,90, το δείγμα θα πρέπει να εξεταστεί με τη βοήθεια συμπληρωματικών δοκιμασιών προκειμένου να επιβεβαιωθεί το αποτέλεσμα. Ένα κατ' επανάληψη αντιδρών δείγμα που έχει επιβεβαιωθεί από συμπληρωματικές δοκιμασίες, θα πρέπει να θεωρηθεί θετικό για αντι-HCV. Στην περίπτωση κατ' επανάληψη οριακών αποτελεσμάτων, συνιστάται η ανάλυση των δειγμάτων επανελέγχου.

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Χαρακτηριστικά απόδοσης

Βαθμονόμηση και ανιχνευσιμότητα της βαθμονόμησης
Η βαθμονόμηση του προσδιορισμού αντι-HCV *Vitros* είναι ανιχνεύσιμη σε έναν εσωτερικό βαθμονομητή αναφοράς στον οποίο έχουν καθοριστεί οι τιμές με τρόπο ώστε να βελτιστοποιείται η κλινική απόδοση ευαισθησίας και ειδικότητας.

Ακρίβεια
Η ακρίβεια εκτιμήθηκε σύμφωνα με το πρωτόκολλο EP5-T2 της Εθνικής Επιτροπής για Κλινικά Εργαστηριακά Πρότυπα[9]. Προσδιορίστηκαν δύο αντίγραφα καθενός από τα 4 δείγματα άπαξ ημερησίως για τουλάχιστον 20 διαφορετικές ημέρες. Το πείραμα πραγματοποιήθηκε με τη χρήση 2 παρτίδων αντιδραστηρίου σε διαφορετικά συστήματα. Τα δεδομένα που παρουσιάζονται είναι αντιπροσωπευτικά για την απόδοση του προϊόντος.

Πίνακας 1: Ακρίβεια

Αντιπροσωπευτικό αποτέλεσμα	Εντός της ανάλυσης		Εντός της βαθμονόμησης		Εντός του εργαστηρίου	
	SD*	CV(%)*	SD*	CV(%)*	SD*	CV(%)*
0,14	0,00336	2,4	0,00835	4,9	0,00945	6,9
2,04	0,044	2,1	0,132	6,4	0,134	6,6
5,74	0,132	2,4	0,339	5,8	0,354	6,2
14,9	0,187	1,2	0,454	3,1	0,576	3,9

*Ρίζα αθροίσματος διαφορών μέσου (RMS)

Εντός της ανάλυσης: Ακρίβεια ανάμεσα στους εις διπλούν προσδιορισμούς, υπολογιζόμενη κατά μέσο όρο για όλες τις αναλύσεις.
Εντός της βαθμονόμησης: Ολική ακρίβεια, με σταθμισμένα συστατικά της απόκλισης εντός της ανάλυσης και μεταξύ των ημερών.
Εντός του εργαστηρίου: Έναμέτρο της επίδρασης της επαναβαθμονόμησης στην ολική ακρίβεια που υπολογίστηκε σε μια παρτίδα αντιδραστηρίου με τη χρήση δεδομένων από 4 βαθμονομήσεις.

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Ευαισθησία
435 δείγματα που είχαν βρεθεί θετικά για το αντι-HCV σε έναν προσδιορισμό ανοσοαποτύπωσης HCV, ελέγχθηκαν με τον προσδιορισμό αντι-HCV *Vitros*. Η ευαισθησία για αυτόν τον πληθυσμό δειγμάτων στον προσδιορισμό αντι-HCV *Vitros* υπολογίστηκε ως 100% (435/435).

Επιπλέον, ελέγχθηκαν 29 πάνελ ορομετατροπής του εμπορίου. Ο προσδιορισμός αντι-HCV *Vitros* έδειξε ίση ή μεγαλύτερη ευαισθησία ορομετατροπής για 29/29 ομάδες, σε σύγκριση με τα δημοσιευμένα αποτελέσματα άλλων προσδιορισμών του εμπορίου.

Ειδικότητα
Δείγματα από 5.374 υποθετικά υγιείς αιμοδότες και 393 κλινικά δείγματα ελέγχθηκαν στον προσδιορισμό αντι-HCV *Vitros* και σε έναν άλλον προσδιορισμό του εμπορίου.

Δείγματα Δείγματα	Αριθμός Δειγμάτων	Αρχικά Αντιδράντα	Κατ' επανάληψη Αντιδράντα	Επιβεβαιωμένα Θετικά
Δότης	5.374	14	13	0
Κλινικά	393	1	1	0

Η ειδικότητα για τον προσδιορισμό αντι-HCV *Vitros* για τα δείγματα αιμοδοτών υπολογίστηκε ως 99,76% (5.361/5.374) με βάση τα κατ' επανάληψη αντιδράντα. Η ειδικότητα για τον προσδιορισμό αντι-HCV *Vitros* για τα κλινικά δείγματα υπολογίστηκε ως 99,75% (392/393) με βάση τα κατ' επανάληψη αντιδράντα.

Επιπλέον, 161 δείγματα από τις ακόλουθες υποομάδες με δυνητικώς διασταυρούμενες αντιδράσεις ελέγχθηκαν στον προσδιορισμό αντι-HCV *Vitros*: CMV θετικό, EBV θετικό, HIV θετικό, μη ιογενείς ηπατικές νόσοι, άλλες ιογενείς ηπατικές νόσοι (π.χ. HBV, HAV), συστηματικός ερυθηματώδης λύκος, ρευματοειδής παράγων θετικός, πρόσφατοι εμβολιασμοί (π.χ. κατά της γρίπης), δείγματα αντιδράντα σε ζυμομύκητες. Από τα δείγματα αυτά κανένα δεν βρέθηκε να είναι ψευδώς αντιδράν στον προσδιορισμό αντι-HCV *Vitros*.

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Ομάδα εργασίας ADM: Περίληψη της αξιολόγησης

Ο προσδιορισμός αντι-HCV *Vitros* αξιολογήθηκε από την ομάδα εργασίας ADM. Ελέγχθηκαν 450 δείγματα από 246 ασθενείς που είχαν μολυνθεί από διαφορετικούς γονοτύπους του ιού της ηπατίτιδας C και 50 δείγματα από την ομάδα HCV SFTS, συμπεριλαμβανομένων 27 RNA αντιδρώντων δειγμάτων. Ο προσδιορισμός αντι-HCV *Vitros* παρουσίασε καλή ευαισθησία για την ανίχνευση πρώιμης ορομετατροπής και για τον έλεγχο άλλων κατηγοριών αντιδρώντων δειγμάτων, συμπεριλαμβανομένων εκείνων που παρουσίασαν μεμονωμένη αντιδραστικότητα και εκείνων που προέρχονταν από χρόνιους φορείς.

Πληθυσμός 2.018 δειγμάτων αίματος από δότες ελέγχθηκαν με τον προσδιορισμό αντι-HCV *Vitros*. Οκτώ δείγματα έδωσαν κατ' επανάληψη αντιδρώντα αποτελέσματα, ένα από τα οποία επιβεβαιώθηκε ως αντιδρών και 6 βρέθηκαν αρνητικά από άλλους προσδιορισμούς. Το αποτέλεσμα του όγδοου δείγματος δεν ήταν δυνατό να ερμηνευθεί (απροσδιόριστο NS3, αρνητικό PCR). Η ειδικότητα για τον προσδιορισμό αντι-HCV *Vitros* ήταν περίπου 99,70%.

Δήλωση περί άδειας παρασκευής

Τα ανασυνδυασμένα αντιγόνα HCV τα οποία χρησιμοποιούνται στον προσδιορισμό αντι-HCV *Vitros*, παρασκευάζονται από τη Chiron Corporation βάσει άδειας εκδοθείσας στις ΗΠΑ και συμφωνίας κοινής παραγωγής.

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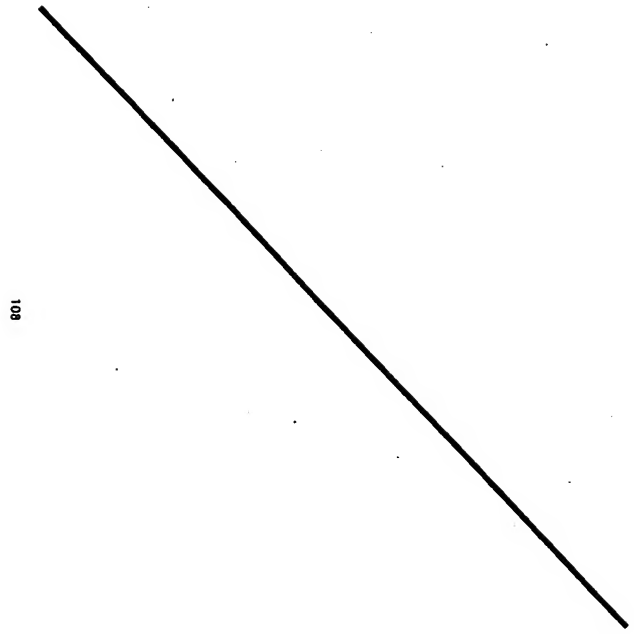

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PT Condições de fornecimento: todo fornecimento é feito de acordo com os termos e condições padrão da Ortho-Clinical Diagnostics ou seus distribuidores. Se solicitado, a Ortho-Clinical Diagnostics disponibiliza cópia dos mesmos.

ES Condiciones de suministro: todos los suministros se ajustan a los términos y condiciones standard de Ortho-Clinical Diagnostics o de sus distribuidores. Se pueden solicitar copias de éstos.

DA Leveringsbetingelser: alle leveringer er underlagt standardbetingelser og -forudsætninger fra Ortho-Clinical Diagnostics eller deres forhandlere. Kopier deraf kan fås på anmodning.

SV Leveransvillkor: all leverans sker i enlighet med standardtermerna och standardvillkoren för Ortho-Clinical Diagnostics eller dess distributörer. Kopior av dessa tillhandahålls på begäran.

EL Οροι προμήθειας: Ολες οι προμήθειες εξαρτώνται από τους πρότυπους όρους και προϋποθέσεις της Ortho-Clinical Diagnostics ή των διανομέων τους. Αντίγραφα αυτών είναι διαθέσιμα όταν ζητηθούν.

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